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Suppressive effects of dichloromethane fraction from the *Areca catechu* nut on naloxone-precipitated morphine withdrawal in mice

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Abstract

In the present study, we investigated the effect of the dichloromethane fraction from *Areca catechu* nut on the severity of naloxone-precipitated morphine withdrawal in morphine-dependent mice. A single intraperitoneal injection of dichloromethane fraction at dose of 125 and 175 mg/kg significantly delayed the onset of withdrawal jumping behavior in a concentration-dependent manner compared to that of saline controls. The dichloromethane fractions also significantly decreased jumping numbers and faecal and urinary excretions during the withdrawal period.

Keywords: *Areca catechu*; Morphine withdrawal; Antidepressant; Naloxone; Jumping

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1. Introduction

Areca nut (*Areca catechu*) is commonly used as an ingredient of betel quid, which also includes leaf of the creeping vine piper betel and lime with or without tobacco. Betel quid chewing has been popular, especially in many Southeastern Asian countries [1,2]. In Taiwan, betel quid is chewed by a large number of both genders [3], probably because this oral habit is more socially accepted than other types of drug abuse. It is estimated that areca nut is chewed by approximately 10% of the world population [4].

Mostly, it is consumed for masticatory and psychoactive purposes [5]. It has been proven that addiction can be induced following prolonged chewing [6].

Whole nut consumption is related to an increased risk of oral submucous fibrosis in human [7–9]. Aqueous extract of areca nut was also demonstrated to induce submucous fibrosis in animal models [10,11]. Especially, when combined with smoking and alcohol drinking, betel quid chewing has about a 100-fold risk of oral cancer compared to that of subjects without this oral habit [12]. Areca nut and betel quid are both on their own also carcinogenic [13]. Thus, isolation of specific ingredients from areca nut with selective actions may contribute more benefits.

It has been previously shown that among various alkaloid constituents from areca nut, alkaloids in dichloromethane fraction were found to be biologically active both in vivo and in vitro. This fraction potently inhibits monoamine oxidase-A activity and thus restores or increases bioavailability of monoamines, 5-hydroxytryptamine or noradrenaline in the brain. Additionally, forced swimming and tail-suspension tests supported that the dichloromethane fraction has antidepressant activity [14].

Chronic administration of opiate substance produces tolerance and dependence. Abrupt cessation of opiate administration results in withdrawal syndrome [15]. Conventionally, methadone, a μ receptor agonist, has been used to relieve withdrawal signs [16]. However, methadone also produces side effects and withdrawal by itself [17–19]. Some antidepressants and other non-opiate substances have been found and used for prevention of withdrawal syndrome. Classical antidepressants such as fluvoxamine or sertraline were found to reduce opioid withdrawal syndrome [20]. In addition, venlafaxine was also demonstrated to attenuate morphine dependence and withdrawal [21]. According to the antidepressant-like activity of the dichloromethane fraction, we hypothesized that the fraction has therapeutic effect, especially for reducing the withdrawal syndrome in drug-addicted patients.

Thus, the present study was aimed to determine whether the dichloromethane fraction from *A. catechu* nut has suppressive effect on withdrawal signs in morphine dependent mice. Effects of the dichloromethane fraction on morphine withdrawal were determined by evaluating jumping behavior, faecal and urinary excretions. These specific behavioral signs reflect the severity of withdrawal syndrome [22,23].

2. Experimental

2.1. General

The following drugs were used: morphine sulphate (Zentiva, SK), naloxone (Sigma, Germany), imipramine (Sigma, Germany) and fluvoxamine (SUN, India).

The drugs were dissolved in normal saline and given to animals in a volume of 5 ml/kg.

2.2. Plant material

A. catechu nuts, purchased from a local market in the Province of Songkhla, Thailand, were identified by Dr. Niwat Keawpradub, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. The dichloromethane fraction was isolated as previously described [24].

2.3. Animals

Male Swiss albino mice (30–35 g) used in this study were bred at the animal house of the Prince of Songkla University. They were housed in a group of 10 mice per cage and maintained under 12/12 dark/light cycle and controlled temperature (22 °C). Standard commercial food pellets and water were available ad libitum. They were acclimatized to these conditions for at least 1 week prior to the experimental use. The experimental protocols described in the present study were approved and guided by the Animals Ethical Committee of the Prince of Songkla University for care and use of experimental animals.

2.4. Development of morphine dependence

Mice were rendered dependent on morphine using the method previously described [25]. Briefly, morphine sulphate was injected (s.c.) three times daily (8.00, 12.00 and 16.00) at 50, 50 and 75 mg/kg, respectively, for 3 days. On day 4, only a single morning dose of morphine (50 mg/kg) was injected before naloxone injection.

2.5. Observation of morphine withdrawal

Withdrawal signs were precipitated by injection of naloxone (1.5 mg/kg, i.p.) 2 h after the injection of morphine. Immediately after naloxone injection, animals were placed individually on filter paper in an observable cylindrical plastic (15 cm in diameter and 50 cm in height). Behaviors of animals were recorded by using digital video camera. Faecal material and urine excreted during a 30-min period of withdrawal were measured.

2.6. Drugs and dichloromethane fraction treatments

One hour before the administration of naloxone, mice were treated with normal saline or injected intraperitoneally with the dichloromethane fraction at dose of 75, 125 and 175 mg/kg. Two other groups were treated with imipramine (20 mg/kg), or fluvoxamine (20 mg/kg).

2.7. Statistical analysis

Experimental data were expressed as mean values ± SEM of the numbers of jumping and weights of faecal material and urine during withdrawal period. Differences were

determined using multiple comparisons versus control group (Dunn's method). Differences with $P \leq 0.05$ were considered statistically significant.

3. Results and discussion

Numbers of jumping were counted and analyzed minute-by-minute. All groups exhibited jumping behavior immediately following naloxone injection. Fig. 1 shows that imipramine and fluvoxamine exhibited relatively equal onsets (53.7 ± 3.9 and 63.6 ± 4.4 s, respectively) to that of saline (56.3 ± 9.5 s). The dichloromethane fraction delayed the onset period in dose-dependent manner. In particular, the 125 and 175 mg/kg exhibited significant longer onsets with 107.1 ± 9.4 and 149.8 ± 22.6 s, respectively.

Also, the total number of jumping over a 5-min period was reduced by the treatment with 125 and 175 mg/kg of the dichloromethane fraction (Fig. 2).

Values of faecal material and urine excreted during a 30-min period of withdrawal are expressed in g and reported in Fig. 3. Compared to saline, all doses of dichloromethane fraction significantly decreased both faecal and urinary excretions, whereas imipramine and fluvoxamine did not show any significant effect.

The present results demonstrate that the dichloromethane fraction is effective in alleviating the incidence of withdrawal jumping in morphine-dependent mice. Jumping is one of the most common signs used to assess the severity of morphine withdrawal [22,26]. Defecation or diarrhea is also commonly found during opiate withdrawal [27,28].

These results also support the previous findings which concluded that the dichloromethane fraction inhibits MAO-A and act as an antidepressant [29]. With this activity, the fraction could increase bioavailability and enhance neurotransmission of monoaminergic, serotonergic and noradrenergic systems in brain. Activation of these systems has been found to reduce the severity of opiate withdrawal [30–32]. Thus, it is possible that the dichloromethane fraction may activate at least one of these systems to affect morphine withdrawal.

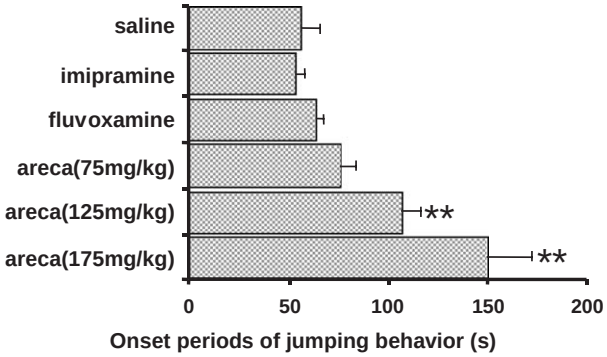

Fig. 1. Effects of dichloromethane fraction from *A. catechu* nut on the onset of naloxone-induced jumping behavior in morphine-dependent mice. The onset periods were measured immediately after naloxone injection until the first jumping. Each group had 7 to 12 mice. Data are means ± S.E.M. $^{**}P < 0.01$ different from the control group.

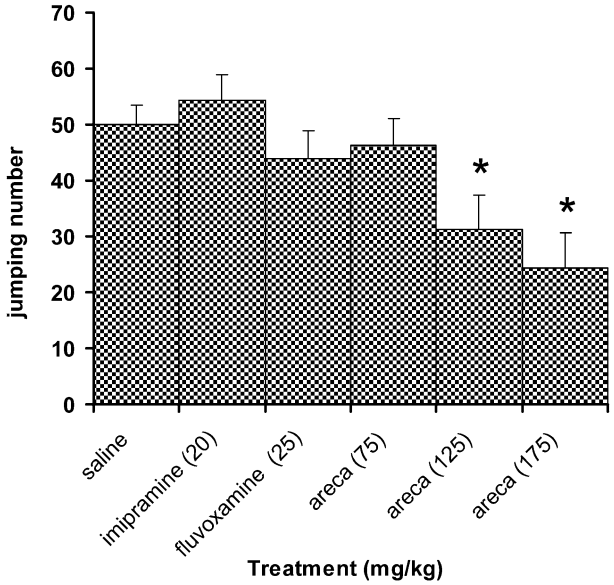

Fig. 2. Effects of dichloromethane fraction from *A. catechu* nut on total number of jumping during 5-min period of morphine withdrawal. Data are means ± S.E.M. $*P<0.05$ different from the control group.

In conclusion, the alleviating effects of the dichloromethane fraction on both signs strongly confirm the promising property of the fraction for clinical purposes. Further studies are needed to investigate the central mechanism and identify specific sites of action of this fraction in the brain.

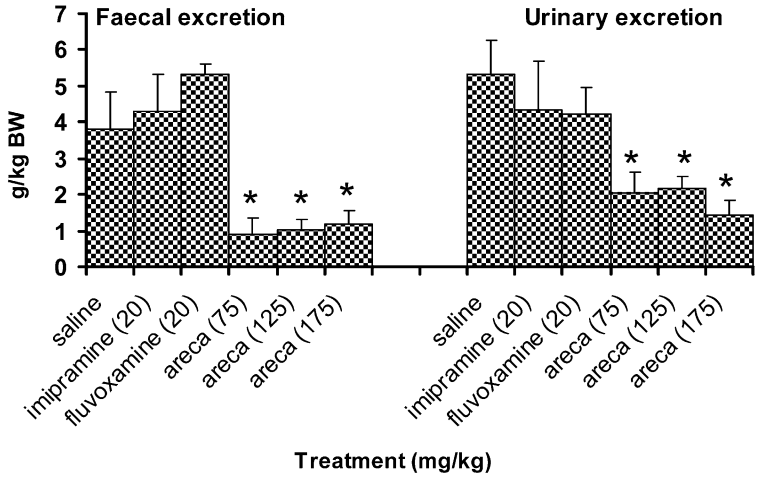

Fig. 3. Effects of dichloromethane fraction from *A. catechu* nut on faecal and urinary excretions in morphine-dependent mice induced by naloxone injection. Weights of faecal material and urine excreted during a 30-min period of morphine withdrawal were measured. Data are means ± S.E.M. $*P<0.05$ different from the control group.

Acknowledgements

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