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EXPERIMENTAL STUDY

# Effect of moxa smoke produced during combustion of Aiye (*Folium Artemisiae Argyi*) on behavioral changes in mice inhaling the smoke

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## Abstract

**OBJECTIVE:** To evaluate the effects that inhalation of the combustion products of the traditional Chinese herb Aiye (*Folium Artemisiae Argyi*) has on the central nervous system.

**METHODS:** Forty Kunming mice (half male) were randomly assigned ($n$ = 10/group) to a control group (C) or one of three moxa smoke concentration groups (% opacity): low (L1; 0.4% ), medium (M1; 2% ), and high (H1; 15% ). Mice in the latter three groups were exposed to moxa smoke in a dynamic gas exposure cabinet for 20 min per day for 7 days. Mice in control group were placed in the same cabinet without any intervention. For the sleep experiments, another 50 mice were divided into five groups of 10 mice each: a saline-injected control group, L1 + pentobarbital sodium (PS)-injected group, M1 + PS group, H1 (15%) + PS group, and a positive control group (10 mg/kg, chlorpromazine, p.o.). The weight, general activities, locomotor activities, rotarod performance, sleep duration, and sleeping rate induced by a subthreshold dose of pentobarbital sodium were recorded in the mice, and the composition of moxa smoke was analyzed using headspace gas chromatography (GC-HS).

**RESULTS:** A low concentration of smoke significantly decreased the frequency of locomotor activities and the time for which the mice remaining on the rotarod; however, a high smoke concentration significantly prolonged the pentobarbital-induced sleeping time and sleeping rate.

**CONCLUSION:** The concentration-dependent relaxing effects of moxa smoke on the Central Nervous System (CNS) were confirmed. Moreover, GC-HS analysis showed that the component present in the highest concentration in moxa smoke was eucalyp-

tol, an essential oil well recognized for its soothing effects on the CNS. This may therefore be accountable for the sedative effects of moxa smoke.



**Key words:** Artemisia Argyi; Moxa smoke; Central nervous system; Inhalation

## INTRODUCTION

Aiye (*Folium Artemisiae Argyi*) is a herbal medicine and used in Tradtional Chinese Medicine (TCM) in moxibustion treatment. Several clinical reports show that moxibustion therapy improves mood[1] and cures mental or nervous system diseases,[2] such as insomnia, depression,[3] neurasthenia,[4] pain,[5] and so forth.[6,7]

Moxa smoke produced from the burning herb has some volatile fragrant substances[8] that play an important role in inhibiting the release of excitatory neurotransmitters via scavenging radicals[9] and antioxidation. We inferred that the pharmacological effects of moxa smoke inhalation might contribute to the tranquilization and calming properties of moxibustion. The combustion of Aiye (*Folium Artemisiae Argyi*) can producesome volatile oils, including aromatic chemicals such as cineole, camphor, borneol, azulene, and caryophyllene, besides those present in fragrant plants with essential oils, such as basil, rosemary, and eucalyptus. In a previous study,[10] we qualitatively analyzed moxa smoke using solid-phase microextraction/gas chromatography-mass spectrometry (SPME- GC-MS) and showed that its chemical composition 10-40 min after burning was mainly aromatic compounds, which could be used in the flavor and fragrance industry. Aromatic substances[11] are known to relax the central nervous system (CNS) by calming the brain through the olfactory system.[12] However, the effects of moxa smoke inhalation on the CNS is as yet unknown.

In the present study, we aimed to investigate potential sedative effects of moxa smoke on mice who took the smoke by inhalation. And explore the possible mechanisms underlying the actions of the moxa smoke.

## MATERIALS AND METHODS

### *Materials*

Aiye (*Folium Artemisiae Argyi*) was plucked on June 16, 2010 (day of Chinese Traditional Dragon Boat Festival), at Qichun, Hubei Province (area of origin), and was identified by a professional pharmacist from the Institute for Biochemical Drug Control, Beijing. It was dried in the shade, sealed for preservation, and made into *Qi* moxa (proportion of 3∶1). Pentobarbital sodium (PS; content ≥ 95.0%) and chlorpromazine (10 mg/kg) were obtained from Shanghai Chemical Reagent Co., Ltd., while sodium chloride injection (0.9%, specification of 100 mL: 0.9 g) was obtained from Shandong Golden Ocean Pharmaceutical Co., Ltd. (lot number: 20120806). The HOPE-MED8050 smoke inhalation device was purchased from Tianjin Hepu Development Area Industry & Trade Co., Ltd.; the YLS-4C rotarod system, from Zhenghua Biological Instrument Equipment Co., Ltd. (Huaibei, Anhui Province); and the YLS-1B mouse autonomic activities recorder, from the Academy of Medical Sciences, Shandong Province. The DB-5MS capillary column (W = 30 m, H = 0.25 mm, L = 0.25 μm ).

### *Animal studies*

Two batches of KM mice, both supported by Weitonglihua Animal Center, were used in this study: one had 40 individuals [certification number: SCXK (Jing) 2006-0009] and the other had 50 individuals [certification number: SCXK (Jing) 2006-0011]. The mice were 8 weeks old, half were male, and their weights ranged between 18 and 22 g. They were housed under standardized conditions (on a standard diet, room temperature: 18 ℃-22 ℃, relative humidity: 50%-60%, light-dark rhythm: 12 h cycle). Mice from the first batch were randomly assigned ($n$ = 10/group) to a control group (C) or one of three moxa smoke concentration groups (% opacity): low (L1; 0.4% ), medium (M1; 2%), and high (H1; 15%). Mice from the second batch were divided into five groups ($n$ = 10/group): a saline-injected control group, L1 (0.4%) + PS-injected group, M1 (2%) + PS group, H1 (15%) + PS group, and a positive control group (10 mg/kg chlorpromazine, p.o.). The study was approved by the experimental animal ethics committee of Beijing University of Chinese Medicine.

### *Smoke inhalation*

The smoke inhalation device[13] had six parts: internal cavity, external cavity, heating or cooling system, gas system, operating system, and host computer control system. After the gas generator was started, the following parameters were set: temperature (20 ℃-22 ℃), humidity (45%-63% ), pressure (− 70 kPa), oxygen (26%), and time (20 min). Then, moxa smoke was introduced into the internal cavity when its concentration reached the appropriate levels (0.4% , 2% , and 15%) and remained constant, representing the opacity of smoke. Next, the mice were placed in the internal cavity and exposed to the corresponding concentration of smoke for 20 min a day for 7 days. The equipment was controlled in real time in order to monitor the experimental conditions (Figure 1).

The smoke inhalation device is gas exposure instrument Hope-Med 8050. Moxa smoke was introduced into the Smoke generator when its concentration reached the appropriate levels and remained constant,