Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 15 (2008) 1117–1124
Phytomedicine
www.elsevier.de/phymed

Effects of extracts and neferine from the embryo of *Nelumbo nucifera* seeds on the central nervous system

Yumi Sugimoto[a,*], Sachiko Furutani[b], Atsuko Itoh[c], Takao Tanahashi[c], Hiroshi Nakajima[d], Hideo Oshiro[e], Shujian Sun[e], Jun Yamada[a]

[a]*Laboratory of Pharmacology, Department of Clinical Pharmacy, Yokohama College of Pharmacy, Matano-cho, Totsuka-ku, Yokohama 245-0066, Japan*
[b]*Department of Pharmacology, Kobe Pharmaceutical University, Motoyamakita-machi, Higashianda-ku, Kobe 658-8558, Japan*
[c]*Department of Organic Chemistry, Kobe Pharmaceutical University, Motoyamakita-machi, Higashianda-ku, Kobe 658-8558, Japan*
[d]*Osaka City University, Research Center for Industry Innovation, Sugimoto, Sumiyoshi-ku, 558-8585 Osaka, Japan*
[e]*Shanghai University of Traditional Chinese Medicine, Japan 1-7-20, Nishitenma Kita-ku, Osaka, Japan*

Abstract

The effects of embryos of the seeds of *Nelumbo nucifera* on the central nervous system were studied in mice. MeOH extracts of embryos of *Nelumbo nucifera* seeds significantly inhibited locomotor activity in mice. The MeOH extract was successively partitioned between H_2O and *n*-hexane, between H_2O and $CHCl_3$, and between H_2O and *n*-BuOH. $CHCl_3$ extracts strongly inhibited locomotor activity in mice, although other extracts had no effect on locomotor activity. The main alkaloid of $CHCl_3$ extracts, neferine, dose-dependently inhibited locomotor activity in mice. Neferine induced hypothermia in mice and apparently potentiated thiopental-induced sleeping time. An anxiolytic, diazepam, decreased locomotor activity, rectal temperature and enhanced sleep elicited by thiopental, similar to neferine. In addition, neferine and diazepam showed anti-anxiety effects in the elevated plus maze test. Neferine did not affect muscle coordination by the rota-rod test. Neferine did not affect strychnine- nor picrotoxin-induced seizure. In contrast, diazepam had apparent muscle relaxant and anti-convulsant effects. These results suggest that neferine has several central effects and that neferine may participate in the efficacy of the sedative effects of embryos of the seeds of *Nelumbo nucifera*. The mechanisms of the sedative effects of neferine are not similar to those of diazepam.

Keywords: *Nelumbo nucifera*; Bisbenzylisoquinoline alkaloids; Neferine; Sedation; Behavior; Central nervous system

Introduction

"Lian zi xin", embryo loti (embryo of the seeds of *Nelumbo nucifera* Gaertner, Nymphaeaceae), has been used in Chinese traditional medicine as a sedative, antipyretic and hemostat agent (Chiang Su New Medical College, 1978), indicating that it may possess central effects. A previous study reported that bisbenzylisoquinoline alkaloids, such as neferine and liensinine, were isolated from this plant (Furukawa, 1966); however, the central effects of the embryo of the seeds of *Nelumbo nucifera* have not been characterized. Furthermore, it has not been clarified whether the alkaloid involved in this plant contributes to the central effects.

Out of interest in the sedative activity of this herbal medicine, we reexamined the chemical constituents of the embryo of the seeds of *Nelumbo nucifera* Gaertner

*Corresponding author. Tel.: +81 45 859 1300; fax: +81 45 859 1301.
E-mail address: yumisugi@hamayaku.ac.jp (Y. Sugimoto).

0944-7113/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2008.09.005

and examined the locomotor activity effects of various extracts of embryo loti in mice. Furthermore, to clarify the central effects, we examined the effects of neferine, which is the main alkaloid of $CHCl_3$ extracts, on locomotor activity, rectal temperature, thiopental-induced sleeping, muscle coordination and drug-induced seizures. Furthermore, we examined the effects of nerefine in the elevated-plus maze test to evaluate anti-anxiety effects.

Material and methods

General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter (Japan). UV spectra were recorded on a Shimadzu UV-2500PC spectrophotometer (Shimadzu, Japan) and IR spectra on a Shimadzu FTIR-8200 spectrophotometer (Japan). ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Varian VXR-500 spectrometer (USA) with TMS as an internal standard. Mass spectrometry was obtained with a Hitachi M-4100 mass spectrometer (Japan). Glycerol was used as the matrix for SIMS. TLC was performed on precoated Kieselgel $60F_{254}$ plates (Merck, Germany).

Plant materials

Embryos of the seeds of *Nelumbo nucifera* Gaertner were purchased from Longhua Hospital Shanghai University of Traditional Chinese Medicine in Shanghai, China. A voucher specimen was deposited in the laboratory of Kobe Pharmaceutical University.

Extraction and isolation

Embryos of the seeds of *Nelumbo nucifera* (1200 g) were extracted with hot MeOH. The extracts were evaporated *in vacuo* and the resulting residue (232.6 g) was resuspended in H_2O and extracted successively with *n*-hexane, $CHCl_3$ and *n*-BuOH. The extraction diagram is shown in Fig. 1.

The residue from the $CHCl_3$ layers (8.0 g) was purified by a combination of silica gel CC ($CHCl_3$–MeOH) and preparative TLC ($CHCl_3$–MeOH, 19:1, 9:1; $CHCl_3$–MeOH–NH_4OH, 95:4.5:0.5, 90:9:1; C_6H_6–AcOEt–Et_2NH, 7:2:1) to afford pronuciferine (33.7 mg), thalifoline (6.7 mg), neferine (2.99 g, Fig. 2), liensinine (57.4 mg) and isoliensinine (20.3 mg). The isolated compounds were identified by comparison of their spectral data with those described in the literature.

Animals

Male ICR mice weighing 25–30 g were purchased from SLC Japan (Japan). Mice had free access to food and water, and were maintained on a 12 h dark/light cycle in a room with controlled temperature ($23 \pm 1\ ^{\circ}C$) and humidity ($55 \pm 5\%$). Experiments were performed in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Treatment of extracts and neferine

Extracts of embryos of the seeds of *Nelumbo nucifera* were suspended in 1% carboxylmethyl cellulose-Na and administered p.o. Neferine HCl was dissolved in saline and given i.p. or p.o.

Drugs and treatment

Diazepam and thiopental Na (Wako, Japan) were dissolved in saline and injected i.p. Strychnine nitrate (Wako, Japan) or picrotoxin (Wako, Japan) was dissolved in saline and given s.c.

Locomotor activity

The locomotor activity of animals during the testing period was counted by an activity sensor (NS-AS01, Neuroscience Inc., Japan). Experiments were performed 60 min after administration of extracts or drugs.

Rectal temperature

Rectal temperature was measured by a thermometer (BAT-12, Sensortek, USA). The thermister probe was inserted 2 cm into the rectum.

Thiopental Na-induced sleep

Thiopental Na at 60 mg/kg was injected i.p. 15 min after the administration of neferine or diazepam. The onset time and duration of loss of the righting reflex were recorded.

Elevated plus maze test

The elevated plus maze (method of according to Pellow et al., 1985; Lister, 1987) consisted of two closed arms ($10 \times 50 \times 40$ cm) and two open arms (10×50 cm) emanating from a common central platform (10×10 cm). The closed and open arms were arranged opposite to each other. Thirty minutes after the injection of neferine