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Gomisin N isolated from *Schisandra chinensis* augments pentobarbital-induced sleep behaviors through the modification of the serotonergic and GABAergic system

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ABSTRACT

The fruits of *Schisandra chinensis* have been used for the treatment of insomnia in oriental countries for more than thousands of years. However, the pharmacological properties and the mechanism of sedative and hypnotic effects have not yet been studied. Gomisin N is one of the major bioactive constituents from the fruits of *Schisandra chinensis*, and in this paper we reported a detailed study on the effects and mechanisms of Gomisin N on its sedative and hypnotic activity for the first time. These results implied that Gomisin N possessed weak sedative effects on locomotion activity in normal mice, and produced a dose-dependent(5–45 mg/kg, i.p.) increase in sleep duration in pentobarbital-treated mice, thus, itself did not induce sleep at higher dose which was used in this experiment (45 mg/kg, i.p.). It also can reverse the rodent models of insomnia induced by *p*-chlorophenylalanine (PCPA) and caffeine, which could exhibit a synergistic effect with 5-hydroxytryptophan (5-HTP) as well; furthermore, the hypnotic effects of Gomisin N were inhibited by flumazenil (a specific $GABA_A$-BZD receptor antagonist). Altogether, these results indicated that Gomisin N produced beneficial sedative and hypnotic bioactivity, which might be mediated by the modification of the serotonergic and GABAergic system.

1. Introduction

Schisandra chinensis (Trucz.) Baill. (Schisandraceae) has been regarded as a useful material in the regulation of various pathological conditions over the last several decades in Asian countries [1]. According to the traditional Chinese medicinal theories, the fruits of *Schisandra chinensis* are defined as a tonic for the yin and blood, especially, to be used in cases of kidney yin deficiency. Therefore, it was also recorded for treating asthmatic cough, spontaneous or night sweats, insomnia etc. in pharmacopoeias.

Previous reports suggested that the major bioactive constituents of *Schisandra chinensis* are lignans belonging to the dibenzocyclooctane type, and more than 40 lignans have been isolated from this fruit [2–4], including Gomisin N, schizandrin C, schizandrin, Gomisin B and so on. Previous information indicated that the extract of *Schisandra chinensis* rich with bioactive dibenzocyclooctane lignans may be useful in the prevention and treatment of Alzheimer's disease [5], and the ethanol fraction of Fructus Schisandrae fruit possesses potent sedative and hypnotic activity [6]. The lignan-rich fractions of Fructus Schisandrae can improve insulin sensitivity via the PPAR-γ pathways in in vitro and in vivo studies [7]. Furthermore, pharmacological studies of lignans isolated from *Schisandra chinensis* have also revealed anti-cancer properties, anti-hepatocarcinogenesis, antioxidant and anti-inflammatory activities [8–12]. As one of the main constituents from *Schisandra chinensis*, Gomisin N was reported to possess a

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series of bioactivity, such as it may be a useful chemotherapeutic agent to induce the apoptosis of U937 cells through a signaling cascade of mitochondria-mediated intrinsic caspase pathways [13], which also demonstrated that Gomisin N is involved in the hepatoprotective effect of the FSC extract, which has therapeutic potential for liver disease [14]. Gomisin N may serve as a novel Wnt/b-catenin inhibitor for the prevention and treatment of human colorectal cancers [15] etc.; however, up to the present, there is still no report about the action and the mechanism of sedative and hypnotic for Gomisin N, which is very important for developing a new generation of hypnotic drugs.

In this paper, the sedative and hypnotic activities of Gomisin N have been investigated. The mechanism and characters of sedative–hypnotic actions of Gomisin N have also been explored on different models of insomnia mice. Finally, we propose a mechanism for the sedative and hypnotic effects of Gomisin N with respect to its potential for treating insomnia.

2. Materials and methods

2.1. Animals

Adult male KunMing mice (weighing 25 ± 2 g) were obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China), The animals were housed in acrylic cages (20 × 35 × 17 cm) with water and food available ad libitum under an artificial 12-h light/dark cycle (light from 5:00 a.m. to 5:00 p.m.) in a sound-proof room (25 ± 1 °C) with food and water available *ad libitum* for the duration of the study. Experiments were carried out in compliance with the National Institutes of Health and institutional guidelines for the humane care of animals and were approved by the Animal Care Committee of Shenyang Pharmaceutical University. Every effort was made to minimize the number of animals used and any pain and discomfort experienced by the subjects.

2.2. Drugs and reagents

Schisandrae fructus were collected from Liaoning Province in China and identified by Professor Ying Jia (Department of Pharmacognosy, Shenyang Pharmaceutical University) according to the guidelines of the Chinese Pharmacopoeia (2010). Gomisin N (purity >99%) was isolated by authors. Other drugs used in this study, flumazenil (FLU), *p*-chlorophenylalanine (PCPA), caffeine, and 5-hydroxytryptophan (5-HTP) were purchased from Melonepharma Co. Ltd (Dalian China), pentobarbital sodium was obtained from Merck Co. Ltd (Shanghai China), and diazepam (DZP) injection was provided by Shenyang Hospital. All other chemicals and reagents were of the highest grade available.

2.3. Bioactive-guided fractionation and isolation of the active Gomisin N

Air-dried and powdered seeds of Schisandrae Fructus (3 kg) were exhaustively extracted with 95% aqueous EtOH (3 × 10 L) at reflux. Each crude extract with different polarities was performed on the sedative and hypnotic neuropharmacological models. The bioactive-guided study revealed a sedative–hypnotic activity of the petroleum ether fraction (200 g) which was subjected to open column chromatography over silica gel (1:10, w/w), eluted with a petroleum ether–EtOAc gradient from 100% to 0% petroleum ether. A total of 10 fractions were collected, Fraction B (28 g) was further separated by silica gel column eluted with petroleum ether–acetone (100:1) to afford two subfractions, and Fraction B-1 was further separated by Semi-Preparative HPLC to afford Gomisin N (300 mg) (Fig. 1), which was identified by comparing its physical and chemical properties with published data.

2.4. Treatments

The animals were acclimatized to the laboratory condition 7 days prior to behavioral study and were maintained in the laboratory until the completion of the study. The pentobarbital-induced sleep test used 45 mg/kg (i.p.) as the hypnotic dose of sodium pentobarbital (with a 100% rate of sleep onset) and 30 mg/kg (i.p.) as the subhypnotic dose (rate of sleep onset <10%). PCPA was suspended in 0.5% gum acacia/physiological saline and administered subcutaneously (300 mg/kg, s.c.). Gomisin N (5, 15 and 45 mg/kg) and DZP (2 mg/kg) were dissolved with 1% dimethyl sulfoxide (DMSO) and FLU(8 mg/kg), caffeine (7.5 mg/kg), and 5-HTP (7.5 mg/kg) were dissolved in physiological saline, and were administered intraperitoneally (i.p. 0.05 ml/10 g). All the drug solutions were freshly prepared before use.

2.5. Behavioral analyses

Behavioral tests were performed in a soundproof room with a neutral environment. All of the tests were carried out between 08:30 and 11:30 or between 13:00 and 13:30, with matching between the groups. The observers were blind to the treatment.

2.5.1. Inner open-field behavior test

The sedative activity was investigated by determining the spontaneous locomotor activity of mice in an open field, animals were placed in an open filed experimental video analysis system (ZS-ZFT, Huaibei Zhenghua Bio-Apparatus Co. Ltd, China). Mice were acclimated to the activity cages individually for 5 min. Twenty five minutes after injection with Gomisin N (5, 15 and 45 mg/kg, i.p.), diazepam (2 mg/kg, i.p.) or 1% DMSO, then, the locomotion activity of each mouse was measured for 5 min. The interruptions of beams of two consecutive infrared sensors were collected for 5 min as a reflection of locomotor activity. After each testing session, the enclosures were thoroughly cleaned with 70% ethanol and

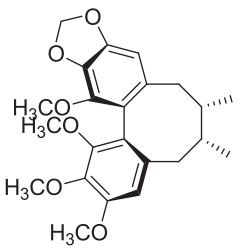

Fig. 1. Chemical structure of Gomisin N.