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Anticonvulsant and sedative–hypnotic activity screening of pearl and nacre (mother of pearl)

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ABSTRACT

Ethnopharmacological relevance: Pearl and nacre are valuable traditional medicines to treat palpitations, convulsions or epilepsy in China for thousands of years. However, the active ingredients are not clear till now.

Aim of the study: The main purpose of the current investigation was to assess the anticonvulsant and sedative–hypnotic activity of pearl powder and nacre powder, including their corresponding 6 protein extracts.

Material and methods: Determination of the amino acid composition of the obtained protein was carried out by ultra-performance liquid chromatography (UPLC) combined with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) pre-column derivatisation. The influence of the tested drugs on locomotor activity and convulsions latency was recorded. The contents of 5-Hydroxytryptamine (5-HT) and γ-aminobutyric acid (GABA) in brain were detected by enzyme-linked immunesorbent assay (ELISA) kits. In addition, immunohistochemistry was carried out to evaluate the changes of 5-HT3 and $GABA_B$. In parallel, the expressions of them were demonstrated by western blot.

Results: The obtained data suggested that pearl original powder (1.1 g/kg), pearl water-soluble protein (0.2 g/kg), pearl acid-soluble protein (0.275 g/kg), pearl conchiolin protein (1.1 g/kg), nacre original powder (1.1 g/kg), nacre water-soluble protein (0.2 g/kg), nacre acid-soluble protein (0.7 g/kg) and nacre conchiolin protein (1.1 g/kg) could down-regulate the expression of 5-HT3 and up-regulate the level of $GABA_B$ to varying degrees compared with the control group. Besides, drug administration also reduced the locomotor activity and increased convulsions latency with a certain mortality.

Conclusions: These findings correlated with the traditional use of pearl and nacre as sedation and tranquilization agents, thus making them interesting sources for further drug development and also providing critical important evidence for the selection of quality control markers.

1. Introduction

Pearl, as recorded in the Chinese Pharmacopeia 2015, is formed in oysters, *Pteria martensii* (Dunker), or mussels, *Hyriopsis cumingii* (Lea) or *Cristaria plicata* (Leach) and nacre (mother of pearl) is the conch of these molluscs (China Pharmacopoeia Committee, 2010). As traditional Chinese medicines (TCMs), both pearl and nacre are recorded as anticonvulsant and sedative–hypnotic agents from the ancient times (Dai et al., 2008; Liu et al., 2014a, 2014b). There are at least 57 Chinese patent medicines (CPMs) containing pearl or nacre in Chinese Pharmacopeia 2015 and most of them have sedative efficacy, such as Angong Niuhuang Pill, Xinnaojing Tablet, Xiegan Anshen Pill, and Qingkailing Injection (Guo et al., 2014, Zhang et al., 2015a). Medicinal pearl and nacre are generally used in the form of original powder in pharmaceutical factory or in clinical, through thoroughly smashing and grinding of natural products to make the ingredients more bioavailable. A considerable number of investigations have revealed that pearl and nacre are rich sources of calcium, accounting for over 90% of the weight, followed by conchiolin protein, and a small amount of trace metal elements. Although the chemical compositions and pharmacological efficacy of the two traditional medicines are clear, the exact bioactive ingredients are still far from identification, which lead to a lower use efficiency of the natural products and also hinder the development of the related novel products.

5-HT is an important excitatory neurotransmitter that widely distributed in animals. It binds with multiple receptors to regulate

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many complex behaviors in vertebrates and invertebrates (Gerhardt and Heerikhuizen, 1997; Tierney, 2001). It is well known that 5-HT is engaged in learning, anxiety, emotion, reproduction, pain perception, sleep and locomotion in mammals. However, it is found that 5-HT and its receptors are implicated in neuronal functions including circadian rhythm, feeding, locomotion, memory, parturition and development (Filla et al., 2004; Kawai et al., 2011; Panasophonkul et al., 2009). Inhibitory neurotransmitter GABA is also one of the critical neurotransmitters (NTs), whose metabolites are closely related to the central nervous system (CNS) depressant activity. In addition, previous literature has documented the role of GABA in CNS of mollusks (Gunaratne et al., 2014). However, it still remains unknown that whether pearl powder, nacre powder and their corresponding protein extracts could improve locomotor activity and convulsions latency. Moreover, the exact active ingredients need to be illustrated. Hence, the current experiment screened the anticonvulsant and sedative–hypnotic activity of pearl powder, nacre powder and their corresponding protein extracts in regulating 5-HT and GABA.

2. Materials and methods

2.1. Materials

Eighteen batches of pearls and 18 batches of nacres were purchased from medicinal plant markets, mainly from Shandong and Zhejiang Provinces in China. The pooled samples from different batches of pearls and nacres were prepared as the tested samples. Voucher specimens were deposited in the laboratory of the author.

Amino acid standards (purity 99%) were purchased from the China Institute of Pharmaceutical and Biological Products. The internal standard (IS), α-amino butyric acid (AABA), was purchased from Sigma (St. Louis, MO, USA) with a purity of 99%. AccQ·Tag™ Eluent A concentrate (P/N 186003838) and Elution B (P/N 186003839) used for gradient program, as well as the AccQ·Fluor™ pre-column AQC derivatisation kit were from Waters (Milford, MA, USA). Diazepam (2 ml/10 mg) was purchased from Tianjin Jinyao amino acids CO., Ltd. 5-HT and GABA ELISA kits were purchased from Elabscience Biotechnology Co., Ltd. Primary antibodies against 5-HT and GABA were produced by Cell Signaling Technology Inc (Beverly, MA, USA). All other chemicals and reagents used for study were of analytical grade and were purchased from approved organizations.

2.2. Sample preparation and amino acid analysis

The crude materials were crushed into very fine powder according to the process method recorded in the China Pharmacopoeia Committee (2010). The sample powder of 100 g was immersed with 10 times of water at 4 °C for 24 h and the solution was filtered. The filtrate was freeze-dried to obtain the water-soluble protein. Ten times of diluted hydrochloric acid was then added to the residue and stirred, and allowed to react at 4 °C for 12 h. The solution was centrifuged at 12,000 rpm for 20 min. The precipitate was freeze-dried to yield the conchiolin protein and the supernatant was dialyzed with the molecular weight cut off (MWCO) 3000 for 48 h and the solution was then freeze-dried to obtain the acid-soluble protein. Amino acid analysis was carried out by UPLC combined with AQC pre-column derivatisation in accordance with the method described previously (Zhang et al., 2015b).

2.3. Animals

100 male and 100 female mice, weighing 20–22 g, obtained from Shanghai Jiesijie Laboratory Animal Co., Ltd., were housed in a temperature and humidity controlled animal facility with a set of 12 h light–dark cycle. Mice had free access to water and food pellets *ad libitum*.

2.4. Locomotor activity test

Mice were randomly divided into ten groups ($n=10$) with half male and female as follows: control group (A), diazepam (2.2 mg/kg) group (B), pearl original powder (1.1 g/kg) group (C), pearl water-soluble protein (0.2 g/kg) group (D), pearl acid-soluble protein (0.275 g/kg) group (E), pearl conchiolin protein (1.1 g/kg) group (F), nacre original powder (1.1 g/kg) group (G), nacre water-soluble protein (0.2 g/kg) group (H), pearl acid-soluble protein (0.7 g/kg) group (I) and nacre conchiolin protein (1.1 g/kg) group (J). The dosage were selected based on the results in the preliminary experiment and the related reference (Dai et al., 2008). After starvation for 12 h, mice were placed individually in an open container (50×20 cm^2) with a black surface covering the inside walls and allowed to explore freely to acclimatize the environment for 2 min before the test. The container was divided into 12 equal squares and the number of panes was recorded. The number of squares crossed and rearing behaviors were recorded for 4 min. The above experimental procedure was repeated once 1 h after intragastrically treated with tested drugs and diazepam.

Then, mice were sacrificed, and the brain was harvested subsequently and set aside at −80 °C for further analysis.

2.5. Anticonvulsant experiment

Mice were randomly divided into ten groups ($n=10$) with half male and female as follows: control group, diazepam group, pearl original powder (1.1 g/kg) group, pearl water-soluble protein (0.2 g/kg) group, pearl acid-soluble protein (0.275 g/kg) group, pearl conchiolin protein (1.1 g/kg) group, nacre original powder (1.1 g/kg) group, nacre water-soluble protein (0.2 g/kg) group, nacre acid-soluble protein (0.7 g/kg) group and nacre conchiolin protein (1.1 g/kg) group. Tested drugs were intragastrically administered for 3 consecutive days. Diazepam (2.2 mg/kg) was orally treated for 3 consecutive days. After intragastric administration 1 h on day 3, mice were intraperitoneally injected with 0.5% of pentylenetetrazol (PTZ, 100 mg/kg). Convulsions latency time was recorded subsequently and mortality of mice were observed 6 h later.

2.6. Measurement of 5-HT3 and $GABA_B$ neurotransmitter levels

The measurements of 5-HT3 and $GABA_B$ in brain of mice were conducted using ELISA kits according to the manufacturer's instruction.

2.7. Immunohistochemistry

The paraffin sample of brain were heated for 1 h in the oven, deparaffinized in xylene, rehydrated by graded ethanol solutions, microwaved in sodium citrate buffer, cooled to room temperature naturally and incubated in 3% hydrogen peroxide. Each section was blocked with 3% BSA at room temperature. Samples were then incubated with primary antibody overnight at 4 °C after removing blocking solution, secondary antibody for 20 min at 37 °C and three antibody for another 20 min at 37 °C, respectively. Then, sections were stained with DAB and restained with hematoxylin. Followed by dehydrating and drying, the sections were mounted with neutral gum and observed under a microscope.