Anxiolytic-like Effects of Methanol Extract of Zizyphi Spinosi Semen in Mice

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Abstract — Zizyphi Spinosi Semen (ZSS), a traditional Chinese folk medicine, has been used for treatment of insomnia and anxiety. This experiment was performed to investigate the anxiolytic-like effect of methanol extract of ZSS (MEZSS) in mice by using the experimental paradigms of anxiety and compared with that of a known anxiolytic, diazepam. In the elevated plus-maze test, it showed that MEZSS (100 mg/kg, p.o.) and diazepam (2.0 mg/kg, p.o.) increased the percentage of time spent on the open arms and the number of open arm entries. MEZSS (50, 100 and 200 mg/kg, p.o.) and diazepam (0.5 mg/kg, p.o.) significantly increased the number of head dips compared with that of control group in the hole-board test. However, MEZSS has no effect on decreasing the locomotor activity, while diazepam (2.0 mg/kg, p.o.) significantly inhibited locomotor activity. MEZSS did not decrease the strength force in the grip strength test, either. In addition, GABAergic involvements were also investigated to understand the possible mechanisms. GABAₐ receptors subunits and glutamic acid decarboxylase (GAD) were not over expressed, compared with that of the saline group. We also found that MEZSS did not increase chloride influx in cultured cerebellar granule cells. It is concluded that MEZSS might have anxiolytic-like effects, but these effects might not be mediated by GABAergic transmission.

Keywords □ methanol extract of Zizyphi Spinosi Semen (MEZSS), anxiolytic-like effect, elevated plus-maze, hole-board, locomotor, grip strength, GABA subunits, GAD, chloride influx.

INTRODUCTION

Anxiety affects one-eighth of the total population worldwide and has become an important area of research interest in psychopharmacology (Eisenberg et al., 1998). Benzodiazepines have been used for the treatment of several forms of anxiety although these compounds have well-known their side-effects such as sedation, muscle relaxation, amnesia and dependence (Jordan et al., 1996). However, many researchers have been evaluated new compounds with less undesirable effects from herbs (Griffiths et al., 1987).

Zizyphi Spinosi Semen (ZSS), the dried seed of Zizyphus jujuba Mill var spinosa (Rhamnaceae), has been known to contain many pharmacologically active components (Peng et al., 2000). It has been used as an analgesic, tranquilizer and anti-convulsant in oriental countries such as Korea and China for over 2500 years (Lee et al., 2005) and has been prescribed for the treatment of insomnia and anxiety in Asia (Lee et al., 2004). Recently it was reported that ZSS significantly increase sleep time induced by pentobarbital (Adzu et al., 2002). It was reported that Sanjoin-Tang showed anxiolytic-like effects and water extract of ZSS also increased open arm entries and spent time in open arms in high doses (Ahn et al., 2004). In this experiment, we are interested in whether the methanol extract of ZSS (MEZSS) might exert anxiolytic-like effects using more experimental paradigms of anxiety. In addition, GABAergic involvements were also investigated to understand the possible mechanisms.

MATERIALS AND METHODS

Animals

Male ICR mice (Samako, Korea) weighing 20-25g, in groups of 10-12, were used throughout the experiments. Animals were housed in acrylic cages (45×60×25 cm) with water and food available ad libitum under an artificial 12-h light/dark cycle (light on at 7:00) and at a constant temperature (22 ± 2 °C). Mice were housed in the departmental room for 1 week...
before testing to ensure adaptation to the new environment.

**Experimental compound and drugs**

ZSS (300 g) were extracted three times in a reflux condenser for 24 h each with 2 L of 70% methanol. The solution was combined, filtered through Whatman No. 1 filter paper, and concentrated using a rotary vacuum evaporator followed by lipopolitization. The yield was about 10% (w/w). The extraction was dissolved in 0.9% physiological saline with 1% carboxymethylcellulose (CMC). Diazepam was purchased from Myung-In Pharm. Co., Ltd. (Kyunggi-Do, Korea) and was dissolved in 0.9% physiological saline before testing. All other chemicals used for molecular experiments were obtained from Sigma Chemical Co. All the experimental compound and drug were orally administered to the animals 30 min prior to the behavior experiment.

**Elevated plus-maze test**

The elevated plus-maze apparatus consists of four arms (30×5 cm) elevated 45 cm above the floor, with each arm positioned at 90° relative to the adjacent arms. The two enclosed arms had 30 cm walls and to facilitate grip on the open arms these included a raised edge of 0.25 cm. Open and closed arms were connected via a central area (5×5 cm) to form a plus sign. The maze floor was constructed of black Plexiglas and the wall of the enclosed arms was constructed of clear Plexiglas (Chen et al., 2003). Four 25-W red fluorescent lights arranged as a cross at 100 cm above the maze were used as the source of illumination and the video camera was suspended above the maze record movements for analysis. Mice were randomly assigned (with a slight adjustment for matched body-weight) to experimental groups. Diazepam was administered orally 30 min prior to the test. MEZSS was administrated orally 60 min prior to the test. Each mouse was manually restrained by the experimenter. When the unrestrained forepaw was brought into contact with the handle, the animals reliably grasp the bar, and the animal is then gently pulled away form the device (Kehl et al., 2000). After each trial, the floor of the apparatus was wiped absolute methanol to remove traces of previous paths. This test session also was recorded with a camera mounted vertically above the hole-board test.

**Locomotor activity**

Since the plus-maze experiment was affected by changes in locomotor activity, an additional experiment was carried out with the specific aim of monitoring the activity. Separately from the experiment above, spontaneous locomotor activity was measured automatically with a tilting-type ambulometer (AMB-10, O’Hara, Japan). Each mouse was placed in the activity cage (20 cm in diameter, 18 cm in height) and after an adaptation period of 10 min, the test compound administration protocol was implemented. Diazepam (2 mg/kg) and MEZSS (25, 50 and 100 mg/kg) were administrated orally 30 min and 60 min prior to the experiment, respectively. Ambulatory activity was measured for 1 hour after oral administration of the agents. Tilting typed locomotor activity was measured.

**Grip strength**

Accordingly, the first 5 sessions of training were limited to handling each animal for 5 min. Then, there were 10 training sessions during which animals were held around the midsection, facing the handle of the grip strength meter (GSM, designed by TSE-Systems and distributed by Scipio, Inc.), and one of the two arms was manually restrained by the experimenter. When the unrestrained forepaw was brought into contact with the handle, the animals reliably grasp the bar, and the animal is then gently pulled away from the device (Kehl et al., 2000). The GSM then measures the maximal force before the animal releases the bar. Each testing session was performed for both forepaws three times 60 min and 30 min after the administration of MEZSS (25, 50 and 100 mg/kg) and diazepam (2 mg/kg), respectively. Means of three trials were calculated.