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Anxiolytic effect of saponins from *Panax quinquefolium* in mice

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Abstract

The anxiolytic effect of the saponins from *Aniliaeea Panax quinquefolium* L. (PQS) was studied in male mice by using a number of experimental paradigms of anxiety and compared with that of the known anxiolytic compound diazepam. Use of the elevated plus-maze test revealed that PQS (50 mg/kg, p.o.) and diazepam (2.5 mg/kg, p.o.) increased the percentage of time and entries spent in open arms. In the light/dark test, PQS (50 and 100 mg/kg, p.o.) and diazepam (2.5 mg/kg, p.o.) prolonged the time spent in the light area. In the hole-board test, PQS (50 and 100 mg/kg, p.o.) and diazepam (2.5 mg/kg, p.o.) significantly increased both head-dip counts and head-dip duration. Both PQS (50 and 100 mg/kg, p.o.) and diazepam (2.5 mg/kg, p.o.) decreased the total fighting time in the isolation-induced aggressive test. Since PQS, in contrast to diazepam, had no effect on locomotion in these tests, its side-effect profile might be considered superior to the benzodiazepines. Thus, the present findings suggest that PQS might be a potential candidate for use as an anxiolytic drug.

Keywords: Anxiolytic; PQS; Elevated plus-maze test; Hole-board test; Light-dark test; Isolation-induced aggressive test

1. Introduction

Ginseng (*Panax ginseng* Meyer) has been used as one of the most valuable natural medicines in China for more than 2000 years. American ginseng (*Panax quinquefolium* L.), a plant native to North America, is now also cultivated and used in many countries. It belongs to the *Panax* genus of the Araliaceae. It has been shown that ginseng administration produces a variety of effects on the central nervous system. For example, ginseng causes behavioral changes in animals, and these changes appear to be related to the regulation of GABAergic transmission (Kimura et al., 1994). Chronic intake of ginseng stabilizes sleep and wakefulness in food-deprived rats (Lee et al., 1990) and the effects of ginseng extract on learning, memory, and physical capacities have also been reported (Petkov and Mosharov, 1987).

It has been shown that *Panax quinquefolium* exerts many beneficial effects similar to ginseng. For example, *Panax quinquefolium* has been widely used in folk medicine for its antioxidant, antilipid peroxidation, antihypoxia and antifatigue properties (Persons, 1986; Fu and Ji, 2003). In animal experiments, modulatory effects of *Panax quinquefolium* on the central nervous system have been observed by Bensky and Gamble (1993). An extract from the leaves and stems of *Panax quinquefolium* has recently been shown to have an anticonvulsant effect in several animal models of seizures (Lian et al., 2005). These effects are thought to be attribute to the major active ingredients, saponins, in *Panax quinquefolium* (PQS). Over the last decade, researchers have found that PQS can exert beneficial effects on the cardiovascular system via its antiischemic, antiarrhythmic, antihypertensive and antioxidative actions (Lu and Sui, 1996). Previous studies have demonstrated that PQS increases the plasma high-density lipoprotein content and decreases the lipid peroxide levels in hyperlipidemic rats (Li et al., 1993). In addition, PQS protects cultured rat cardiac myocytes from oxidative damage (Yang et al., 1992). It has been reported that PQS has a beneficial effect on stress-induced pathophysiological changes in the central nervous system and it has recently been reported that PQS can improve memory impaired by scopolamine, cycloheximide and sodium pentobarbital in the passive avoidance test (Gao et al., 1995). Ginsenoside Rb1 and pseudoginsenoside-F11, components of PQS, can prevent the memory deficits induced by scopolamine in rats (Benishin et al., 1991; Benishin, 1992; Li et al., 1999). These results

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strongly suggest that PQS might be a potential neuroactive principle.

According to the pharmacological profile of PQS, it is reasonable to assume that PQS might have some other neuroactive activities. Therefore, the present study was designed to investigate the anxiolytic effects of PQS by using several anxiety paradigms: the elevated plus-maze test, light/dark test, hole-board test and isolation-induced aggressive test.

2. Materials and methods

2.1. Plant material

The roots of *Panax quinquefolium* L. were collected from the Jilin province of China, and identified by Prof. Qi-Shi Sun (Shenyang Pharmaceutical University).

2.2. Preparation of extracts

The procedures for extraction and isolation of the saponins from the roots of *Panax quinquefolium* L. were as follow. Briefly, the dried roots of the plant (5000 g) were powdered and then extracted with 70% EtOH (50 L) three times (2 h for each time) under reflux. After filtration, excess solvent was removed under reduced pressure. The EtOH extract was suspended in water and defatted with ether followed by partitioning with *n*-BuOH. The combined *n*-BuOH layers were concentrated to dryness. The dried extract was subjected to HPD100 resin column chromatography, washed with water, and eluted with EtOH to afford a total saponins fraction (1645 g).

2.3. Animals

Male Swiss mice (Experimental Animal Center of Shenyang Pharmaceutical University) weighing 18–20 g were used. The mice were housed in groups of five in cages of 28 cm × 20 cm × 16 cm. Food and water were freely available and the room temperature was controlled at 22 ± 2 °C. Each animal was used once in the behavior tests. All animal use procedures were in accordance with the Regulations of Experimental Animal Administration issued by State Committee of Science and Technology of the People's Republic of China on 14 November 1988.

2.4. Drugs

PQS was purified by the Department of Chemistry for Natural Drugs of Shenyang Pharmaceutical University (purity > 95%) and dissolved in distilled water. Diazepam (DZ) was used as a positive control and purchased from Hubei Pharmaceutical Factory (Hubei, China). Diazepam was ultrasonically dispersed in distilled water. Test drugs were orally administered 30 min before the experiments in a volume of 10 mL/kg. Blank control animals were given the corresponding vehicles. All drugs were freshly prepared before each experiment.

2.5. Procedures

2.5.1. Elevated plus-maze test

The elevated plus-maze comprised two open (30 cm × 5 cm × 0.25 cm) and two enclosed (30 cm × 5 cm × 15 cm) arms that radiated from a central platform (5 cm × 5 cm) to form a plus sign. The maze was constructed of black painted wood. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals. The plus-maze was elevated to a height of 40 cm above floor level by a single central support. Four 25 W red fluorescent lights arranged as a cross at 100 cm above the maze were used as the source of illumination (Chen et al., 2003). The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, were counted during a 5 min test period. The percentage open arm entries and percentage open arm time were used as indices of anxiety. A mouse was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received.

2.5.2. Light/dark test

The apparatus consisted of two 20 cm × 10 cm × 14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100 W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box (Lepicard et al., 2000; Guo et al., 2004). The apparatus was cleaned thoroughly between trials. All behavioral recordings were carried out with the observer unaware of the treatment the mice had received.

2.5.3. The hole-board test

The apparatus was composed of a gray wooden box (50 cm × 50 cm × 50 cm) with four equidistant holes 3 cm in diameter in the floor (Moraira et al., 2000). The centre of each hole was 10 cm from the nearest wall of the box. The floor of the box was positioned 15 cm above the ground and divided into squares of 10 cm × 10 cm with a water-resistant marker. An animal was placed in the center of the hole-board and allowed to freely explore the apparatus for 5 min. The total locomotor activity (numbers of squares crossed), and the number and duration of head-dippings were recorded. A head dip was scored if both eyes disappeared into the hole.

2.5.4. Isolation-induced aggressive test

Isolated mice were prepared as described by Guo et al. (2004). Each mouse was isolated in cages of 28 cm × 20 cm × 16 cm for 6 weeks. Isolated mice were prescreened for aggressive behavior prior to the experiment. An intruder mouse was introduced