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Protective Effects of Pseudoginsenoside-F_{11} on Scopolamine-induced Memory Impairment in Mice and Rats

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Abstract

This study assessed the effects of pseudoginsenoside-F_{11}, a component of *Panax quinquefolium* L., on scopolamine-impaired memory performance in mice and rats.

In the one-trial step-down and step-through passive avoidance tests, although pseudoginsenoside-F_{11} used alone did not affect passive avoidance behaviour in naive mice, the latency of avoidance shortened by intraperitoneal scopolamine (2 mg kg^{-1}) was prolonged after intragastric administration of pseudoginsenoside-F_{11} (2 or 4 mg kg^{-1}, for five days) in both test systems in mice. In the water-maze test, in mice, the time taken to locate the platform after administration of pseudoginsenoside-F_{11} was shorter than that after administration of scopolamine (1 mg kg^{-1}, i.p.). In the two-way active avoidance response test, the latency of avoidance was significantly shorter for the pseudoginsenoside-F_{11}- (1·2 or 2·4 mg kg^{-1}, i.g. for five days) and scopolamine-treated group than for the group of rats given scopolamine only (2 mg kg^{-1}, i.p.). The percentage avoidance was also reduced after intraperitoneal injection of scopolamine, but was reversed by administration of pseudoginsenoside-F_{11}.

These results suggest that pseudoginsenoside-F_{11} antagonized the memory dysfunction induced by scopolamine. However, the mechanism of the memory facilitative action of pseudoginsenoside-F_{11} merits further elucidation.

Pseudoginsenoside-F_{11} is a component of *Panax quinquefolium* L. (American ginseng). It was previously isolated from leaves of *P. pseudo-ginseng* subsp. *himalaicus* Hara (Himalayan Panax) and named by Tanaka & Yahara (1978). Usually, the nomenclature of ginsenosides and pseudoginsenosides is derived from the mobility of these saponins in a one-dimensional thin-layer chromatographic system (Kondo et al 1973; Soldati & Sticher 1980). Pseudoginsenoside-F_{11} does not exist in *P. ginseng* C. A. Meyer (Chen et al 1981), but there are relatively large amounts in the leaves of *P. quinquefolium* (Chen 1990). Structurally, pseudo-ginsenoside-F_{11} is a ocotillol-type triterpene saponin, which is different from the dammarane-type triterpene saponins isolated from the roots and leaves of *P. ginseng* C. A . Meyer (Figure 1).

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There have been reports that pseudoginsenoside-F_{11} increased the amplitude of the action potential, the overshooting, the threshold, the maximum diastolic potential, the maximum rate of depolarization and action potential duration at 10% and 50% levels of repolarization in Wistar rat cardiac cells (Chen et al 1995). Pseudoginsenoside-F_{11}

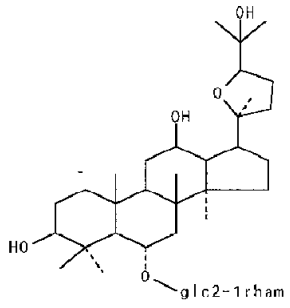

Figure 1. The chemical structure of pseudoginsenoside-F_{11}

significantly elevated blood pressure when injected into the lateral ventricle and posterior hypothalamic nucleus of rats (Yang et al 1994).

It has recently been reported that saponins isolated from *P. quinquefolium* could improve memory impaired by scopolamine, cycloheximide and sodium pentobarbital in the passive avoidance test (Gao et al 1995). Because pseudoginsenoside-F_{11} is a main component of the saponins isolated from *P. quinquefolium*, it is of interest to test whether pseudoginsenoside-F_{11} is an active contributor to this memory-improving action of the saponins. In this study we have used four kinds of behavioural task to investigate the effect of pseudoginsenoside-F_{11} on memory impairment caused by scopolamine.

Materials and Methods

Animals

The study was conducted on male Swiss mice, 20–22 g, and male Wistar rats, 250–300 g. They were housed in plastic cages in groups of five and maintained under standard conditions with a 12 h–12 h light–dark cycle and free access to food and water. The animals were used for the behavioural experiments after they had adapted to laboratory conditions for at least five days.

Drugs

Pseudoginsenoside-F_{11} was isolated from the aerial parts of *P. quinquefolium* L. by the Department of Chemistry for Nature Products of Shenyang Pharmaceutical University. Pseudoginsenoside-F_{11} was dissolved in distilled water and administered intragastrically once a day for five days 60 min before training, at doses of 2 and 4 mg kg^{-1} in mice, and 1·4 and 2·8 mg kg^{-1} in rats. Scopolamine hydrochloride (Sigma, St Louis, MO) dissolved in sterile saline was injected intraperitoneally in a volume of 0·1 mL per 10 g body weight in mice and 0·1 mL per 100 g body weight in rats.

One-trial step-down passive avoidance task

Mice were trained in an apparatus previously described by Yati et al (1991). Naive mice were placed on a platform (4 cm × 3 cm) in a lighted box (12 cm × 10 cm × 30 cm) with a grid floor through which an electric shock of 30 V (50 Hz) was delivered. When the mice stepped off the platform, a constant and continuous electric shock was applied. The normal reaction of the mice was to jump back on to the platform. After 24 h each mouse was again placed on the platform and tested again for step-down latency. The test was conducted 60 min after the fifth oral administration of pseudoginsenoside-F_{11}, 10 min after an intraperitoneal injection of scopolamine 2 mg kg^{-1}.

One-trial step-through passive avoidance task

A one-trial step-through passive task was performed as described by Masanori et al (1995). The apparatus consisted of two compartments, an illuminated box and a dark box separated by a guillotine door. The size of both boxes was 20 cm × 10 cm × 15 cm. During the training, the mouse was placed in the illuminated compartment and allowed to enter the dark compartment through the door. Immediately after entry, a scrambled foot shock (30 V, 50 Hz) was delivered through the grid floor. The mouse could escape from the shock only by stepping back into the safe illuminated compartment. In the test, 24 h after the training, the mouse was again placed in the safe illuminated compartment. The response latency to enter the dark compartment was measured. The latency of mice not entering the dark room during the 300-s observation period was regarded as 300 s. The test was conducted 60 min after the fifth oral administration of pseudoginsenoside-F_{11}, 10 min after intraperitoneal injection of scopolamine 2 mg kg^{-1}.

Water maze task

The maze was a plastic pool (65 cm × 35 cm × 20 cm) filled with clear water to a depth of 10 cm. Water was maintained at 25 ± 2°C. The pool was divided by four pieces of plastic plate into five parts (Figure 2). At the end of the maze there was a platform on to which the mice could climb. During the training, the mouse was put into the water at the starting point and was guided to swim to the end of the maze to climb on to the platform. The mice were trained 60 min after oral administration of pseudoginsenoside-F_{11} and 10 min after scopolamine. Each mouse was trained 10 times daily at intervals of 25 s for five consecutive days.

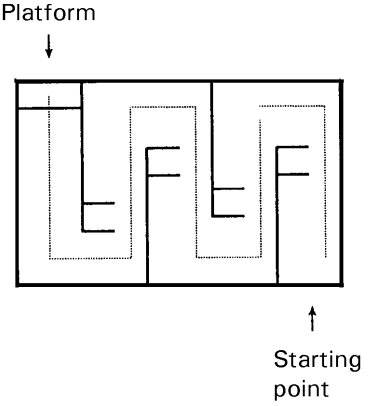

Figure 2. Schematic diagram of the water maze apparatus.

Two-way active avoidance task

The apparatus used in the experiment was a wooden shuttle-box consisting of two identical compartments (30 cm × 24 cm × 41 cm) separated by a vertical wall with a hole, 9 cm in diameter. A 100-W electric light was fixed 30 cm above the hole in each compartment and a separate grid floor made of 0·5-cm diameter brass rods, 1 cm apart, through which a foot shock of 30 V alternating current could be applied. Conditioned stimulus was a flash and unconditioned stimulus was a 30 V foot shock. Both compartments were used as the safe side without the foot shock and as the shock side, in turn, with an interval of 30 s at most.

There were three phases to the procedure: adaptation, training and testing. Rats were adapted to the two compartments for 90 s before the first training trial. The rats were trained to cross the hole from the shock side into the safe side within the duration of 10 s conditioned stimulus, otherwise they were punished with 5 s unconditioned stimulus. Each rat was given 30 trials daily. One trial consisted of 15 s conditioned stimulus followed by 15 s intertrial interval. The last 5 s of conditioned stimulus could overlap with unconditioned stimulus. At the end of each trial the conditioned stimulus and unconditioned stimulus were terminated. An avoidance response was defined as an entry into the safe side before the onset of foot shock. The testing procedure was the same as that used for training. Both training and testing were conducted between 0800 h and 1700 h in the same order (Ma & Yu 1993).

The rats were trained continuously for five days (day 1 to day 5), starting every day 60 min after oral administration of pseudoginsenoside-F_{11}, which was 20 min after intraperitoneal injection of scopolamine 1 mg kg^{-1}. The acquisition of memory was tested two days after day five days of training.

Statistics

Results are expressed as mean ± s.e.m. Differences between individual groups were evaluated by one-way analysis of variance with the minimum acceptable level of significance set at $P < 0.05$.

Results

Table 1 shows the average latency of mice in the step-down passive avoidance test. Pseudoginsenoside-F_{11} administered alone had no significant effect on the memory function in the normal mice. The latency for the scopolamine-treated group was significantly longer than for the vehicle-treated group ($F_{1,16} = 36.1$, $P < 0.001$). However, after administration of pseudoginsenoside-F_{11} 4 mg kg^{-1}, the latency was reduced significantly compared with that for the scopolamine-treated group ($F_{1,14} = 6.3$, $P < 0.05$).

Table 1. Effect of intragastric administration of pseudoginsenoside-F_{11} for five days on memory impairment induced by intraperitoneal scopolamine in the step-down passive avoidance test in mice.

Treatment	Response latency (s)
Saline	168·3 ± 8·0
Pseudoginsenoside-F_{11} 2 mg kg^{-1}	171·5 ± 9·3
Pseudoginsenoside-F_{11} 4 mg kg^{-1}	179·9 ± 8·8
Scopolamine 2 mg kg^{-1}	59·1 ± 20·5†
Pseudoginsenoside-F_{11} 2 mg kg^{-1} + scopolamine 2 mg kg^{-1}	101·0 ± 15·9
Pseudoginsenoside-F_{11} 4 mg kg^{-1} + scopolamine 2 mg kg^{-1}	134·4 ± 21·6*

Data are means ± s.e.m. of results from 8–10 mice. †$P < 0.001$ compared with saline group. *$P < 0.05$ compared with scopolamine group.

In the step-through passive avoidance test, pseudoginsenoside-F_{11} administered alone had no significant effect on memory function in normal mice. The latency for the scopolamine-treated group was significantly longer than for the vehicle-treated group ($F_{1,20} = 84.5$, $P < 0.001$). Pseudoginsenoside-F_{11} at 2 and 4 mg kg^{-1} significantly antagonized the effect of scopolamine ($F_{1,20} = 36.0$, $P < 0.001$ for pseudoginsenoside-F_{11} 2 mg kg^{-1}; $F_{1,20} = 47.9$, $P < 0.001$ for pseudoginsenoside-F_{11} 4 mg kg^{-1}) (Table 2).

Table 3 shows the average time taken by the animals in each group to find the platform. From day 4 to day 5 the mice in the scopolamine group took significantly longer than those in the saline group (on day 4 $F_{1,20} = 5.6$, $P < 0.05$; on day 5

Table 2. Effect of intragastric administration of pseudoginsenoside-F_{11} for five days on memory impairment induced by intraperitoneal scopolamine in the step-through passive avoidance test in mice.

Treatment	Response latency (s)
Saline	290·6 ± 9·2
Pseudoginsenoside-F_{11} 2 mg kg^{-1}	291·1 ± 10·4
Pseudoginsenoside-F_{11} 4 mg kg^{-1}	295·5 ± 10·5
Scopolamine 2 mg kg^{-1}	85·6 ± 19·9†
Pseudoginsenoside-F_{11} 2 mg kg^{-1} + scopolamine 2 mg kg^{-1}	219·7 ± 25·6*
Pseudoginsenoside-F_{11} 4 mg kg^{-1} + scopolamine 2 mg kg^{-1}	251·1 ± 28·4*

Data are means ± s.e.m. of results from 11 mice. †$P < 0.001$ compared with saline group. *$P < 0.001$ compared with scopolamine group.

Table 3. Effect of intragastric administration of pseudoginsenoside-F_{11} for five days on memory impairment induced by intraperitoneal scopolamine in the water-maze test in mice.

Treatment	Time to locate platform (s)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Saline	27·6 ± 1·2	23·1 ± 2·6	20·0 ± 0·8	16·7 ± 0·6	15·6 ± 0·8
Pseudoginsenoside-F_{11} 2 mg kg^{-1}	28·0 ± 1·2	23·6 ± 0·9	19·0 ± 1·2	16·8 ± 0·7	15·5 ± 0·8
Pseudoginsenoside-F_{11} 4 mg kg^{-1}	28·8 ± 1·7	22·0 ± 1·2	18·5 ± 1·2	16·5 ± 0·8	14·3 ± 0·7
Scopolamine 1 mg kg^{-1}	28·6 ± 1·1	22·8 ± 4·1	20·7 ± 4·3	21·2 ± 5·5†	19·9 ± 1·1†
Pseudoginsenoside-F_{11} 2 mg kg^{-1} + scopolamine 1 mg kg^{-1}	28·7 ± 1·2	21·6 ± 0·8	17·7 ± 1·0	15·9 ± 0·8*	16·1 ± 1·3
Pseudoginsenoside-F_{11} 4 mg kg^{-1} + scopolamine 1 mg kg^{-1}	25·9 ± 0·9	22·1 ± 3·4	18·4 ± 2·5	15·3 ± 0·7**	14·9 ± 0·5**

Data are means ± s.e.m. of results from 10–12 mice. †$P < 0·05$ compared with saline group. *$P < 0·05$, **$P < 0·01$ compared with scopolamine group.

$F_{1,20} = 7·6$, $P < 0·05$). However, the longer latency induced by scopolamine was significantly reduced by administration of pseudoginsenoside-F_{11} 2 mg kg^{-1} (on day 4 $F_{1,21} = 7·7$, $P < 0·05$) and 4 mg kg^{-1} (on day 4 $F_{1,20} = 18·1$, $P < 0·01$; on day 5 $F_{1,20} = 11·9$, $P < 0·01$). Pseudoginsenoside-F_{11}, used alone in this dose range, did not affect the time taken by normal animals to find the platform, compared with that taken by saline-treated mice.

The avoidance latencies in the two-way active avoidance test in rats are shown in Table 4. Scopolamine significantly reduced the avoidance latency (on day 4 $F_{1,14} = 6·1$, $P < 0·05$; on day 5 $F_{1,14} = 4·8$, $P < 0·05$; on day 7 $F_{1,14} = 12·3$, $P < 0·01$) and the avoidance percentage (on day 4 $F_{1,14} = 8·0$, $P < 0·05$; on day 5 $F_{1,14} = 6·4$, $P < 0·05$; on day 7 $F_{1,14} = 15·4$, $P < 0·01$) on days 4 and 5 during training and on day 7 during testing. The avoidance latencies reduced by scopolamine from day 4 to day 5 training and day 7 testing were significantly antagonized by administration of pseudoginsenoside-F_{11} 4 mg kg^{-1} (on day 4 $F_{1,15} = 6·0$, $P < 0·05$; on day 5 $F_{1,15} = 7·2$, $P < 0·05$; on day 7 $F_{1,15} = 8·8$, $P < 0·01$). Pseudoginsenoside-F_{11} significantly increased the avoidance percentage reduced by scopolamine (on day 4 $F_{1,15} = 12·5$, $P < 0·01$; on day 5 $F_{1,15} = 14·5$, $P < 0·01$; on day 7 $F_{1,15} = 9·9$, $P < 0·01$) (Table 5).

Discussion

In this study we examined the effect of pseudoginsenoside-F_{11} on memory impairment induced by scopolamine. Our results showed that pseudoginsenoside-F_{11} significantly shortened the latency in the one-trial passive avoidance test. In the active avoidance test, pseudoginsenoside-F_{11} significantly shortened the avoidance latency and increased the avoidance percentage. Moreover, as the experiment progressed, repeated training led to an improvement in reference memory. Thus, the results indicate that working memory and reference memory deficits are ameliorated after administration of pseudoginsenoside-F_{11}.

Table 4. Effect of intragastric administration of pseudoginsenoside-F_{11} for five days on memory impairment induced by intraperitoneal scopolamine in the active avoidance test (expressed as average avoidance latency) in rats.

Treatment	Avoidance latency (s)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
Saline	10·9 ± 0·8	9·3 ± 0·7	7·8 ± 0·8	5·9 ± 0·6	5·4 ± 0·8	4·8 ± 1·1
Scopolamine 2 mg kg^{-1}	12·1 ± 0·9	9·5 ± 1·2	7·0 ± 0·7	8·1 ± 0·6†	8·3 ± 1·0†	9·7 ± 0·8††
Pseudoginsenoside-F_{11} 2 mg kg^{-1} + scopolamine 2 mg kg^{-1}	11·5 ± 0·8	10·2 ± 0·9	7·9 ± 1·1	8·7 ± 1·3	8·8 ± 0·7	8·7 ± 1·2
Pseudoginsenoside-F_{11} 4 mg kg^{-1} + scopolamine 2 mg kg^{-1}	11·4 ± 2·8	7·7 ± 0·5	5·8 ± 0·8	5·7 ± 0·8*	4·5 ± 1·0*	6·4 ± 2·1*

Data are means ± s.e.m. of results from 8 or 9 rats. †$P < 0·05$, ††$P < 0·01$ compared with saline group. *$P < 0·01$ compared with scopolamine group.

Table 5. Effect of intragastric administration of pseudoginsenoside-F_{11} for five days on memory impairment induced by intraperitoneal scopolamine in the active avoidance test (expressed as average avoidance percentage) in rats.

Treatment	Avoidance percentage					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
Saline	44·8 ± 10·6	55·2 ± 8·8	75·2 ± 7·6	82·9 ± 6·8	83·8 ± 6·3	86·7 ± 7·1
Scopolamine 2 mg kg^{-1}	29·6 ± 10·2	62·2 ± 11·8	69·2 ± 5·7	57·8 ± 5·6†	56·3 ± 7·7†	44·4 ± 7·6††
Pseudoginsenoside-F_{11} 2 mg kg^{-1} + scopolamine 2 mg kg^{-1}	38·3 ± 7·2	45·7 ± 9·4	70·4 ± 11·7	59·0 ± 10·7	65·7 ± 6·7	58·1 ± 12·1
Pseudoginsenoside-F_{11} 4 mg kg^{-1} + scopolamine 2 mg kg^{-1}	27·5 ± 8·6	64·2 ± 6·9	80·8 ± 7·9	84·2 ± 4·7*	92·5 ± 3·9*	75·8 ± 6·0*

Data are means ± s.e.m. of results from 8 or 9 rats. †$P < 0.05$, ††$P < 0.01$ compared with saline group. *$P < 0.01$ compared with scopolamine group.

Marked impairment of learning and the memory process was observed for scopolamine-treated mice. The memory-impairing effects of scopolamine have been reported to be a result of disruption of the two major forebrain cholinergic systems. Disruption of the septo-hippocampal system impairs working memory whereas disruption of the cortical cholinergic system affects the reference memory of task performance (Dunnett 1985). In the current study scopolamine significantly prolonged the latencies both in the passive avoidance test and in the active avoidance test and also markedly prolonged the time taken to locate the platform in the water-maze test. However, after administration of pseudoginsenoside-F_{11}, the time taken to locate the platform was significantly reduced compared with that taken by scopolamine-treated mice. These results suggest that pseudoginsenoside-F_{11} improves the deficit of spatial and non-spatial memory by enhancement of the cholinergic system.

The effects of pseudoginsenoside-F_{11} on scopolamine-induced memory dysfunction seem similar to those of the extract of *P. quinquefolium* (Gao et al 1995). In addition to the other constituents in *P. quinquefolium* reported to improve memory function, this is the first observation demonstrating that pseudoginsenoside-F_{11} is one of the main active ingredients in *P. quinquefolium* with memory-enhancing activity.

The mechanism underlying the memory-enhancing effect of pseudoginsenoside-F_{11} is not yet clear. Although it has been reported that pseudoginsenoside-F_{11} effects the central nervous system (Yang et al 1994), the exact mechanism was obscure (Chen et al 1995). Although our results show that pseudoginsenoside-F_{11} antagonized scopolamine-induced memory impairment, one cannot conclude that pseudoginsenoside-F_{11} improves the memory function impaired by scopolamine via direct activation of the central cholinergic system. No study yet conducted has shown that pseudoginsenoside-F_{11} has any cholinomimetic activity in-vivo or in-vitro. The lack of memory-promoting activity of pseudoginsenoside-F_{11} in normal animals might exclude the possibility of direct activation of the cholinergic system by pseudoginsenoside-F_{11}. However, it will be interesting to elucidate further the mechanism of the action of pseudoginsenoside-F_{11} in improving the impaired memory induced by scopolamine.

In conclusion, this study shows that pseudoginsenoside-F_{11} can significantly antagonize scopolamine-induced memory impairment in passive and active avoidance tests. This is the first evidence that pseudoginsenoside-F_{11} is one of the main components of *P. quinquefolium* with memory-enhancing activity. The results suggest that pseudoginsenoside-F_{11} might be a promising compound for clinical use in improving the cognitive impairment caused by central cholinergic dysfunction, such as in patients with senile dementia of the Alzheimer type.

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