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Effects of an aqueous extract of *Puerariae flos* (Thomsonide) on impairment of passive avoidance behavior in mice

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Abstract

The effects of an aqueous extract of *Puerariae flos* (Thomsonide) on ethanol-induced learning and memory impairment and scopolamine-induced amnesia were investigated. Thomsonide exerted an ameliorating effect on the impairment of both memory registration and memory retrieval induced by ethanol. These results indicate that Thomsonide has an antiamnesic effect on the central nervous system in alcoholic intoxication and support the traditional use of *Puerariae flos* for the treatment of alcoholic intoxication. Thomsonide also improved the scopolamine-induced impairment of memory registration in passive avoidance behavior in mice. The results of this study suggest that it may be possible to use Thomsonide for the treatment of age-related memory impairment and dementia.

Keywords: *Puerariae flos*; Isoflavonoids; Learning and memory; Ethanol; Scopolamine

1. Introduction

Pueraria thunbergiana (Leguminosae) is a tendrillar herb that grows widely throughout Southeast Asia, and the root of the plant, Puerariae Radix, has been used in Oriental herbal medicine as an antipyretic and analgesic in the treatment of the common cold (Kim et al., 2003). The dried flower of the plant, *Puerariae flos*, on the other hand, has traditionally been used to treat diabetes mellitus and alcoholic intoxication. The trade mark Thomsonide has been applied to the dried powder obtained from an aqueous extract of *Puerariae flos* that contains large amounts of isoflavonoids and triterpenoid saponins that possess pharmacological activity, and it has been marketed as a commercial food supplement for human health care. Yamazaki et al. (2002) have reported that an extract of *Puerariae flos* promotes elimination of acetaldehyde from the blood of humans, but few extensive pharmacological studies have been conducted to evaluate the antiamnesic effects of *Pueraria thunbergiana* on the central nervous system in alcoholic intoxication. Matsunaga and Mukasa (1986) have reported that mild alcohol intoxication impairs the extremely short-term memory storage process in humans and Bammer and Chesher (1982) have reported observing impairment of retention in mice and rats when ethanol was given before training sessions. Since alcohol consumption particularly impairs learning and memory and *Puerariae flos* has been used in traditional medicine for the treatment of alcoholic intoxication, we investigated the effects of an aqueous extract of *Puerariae flos* (Thomsonide) on learning and memory behavior in mice with retrograde amnesia. In order to clarify the antiamnesic activities of Thomsonide on the passive avoidance behavior of mice, we then designed the present study to evaluate the effects of Thomsonide on learning and memory impairment induced by ethanol and by scopolamine, which induces amnesia that is widely cited as a model for human dementia in general, and for Alzheimer's disease in particular.

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2. Materials and methods

2.1. Materials

Thomsonide (Lot.S-0046709) was purchased from Mikuni Co. (Osaka, Japan). The original powder contained 1.1% water, 13.4% protein, 0.3% fat, 2.2% ash, and 83% carbohydrate, as determined by the manufacturer. According to the results of a high performance liquid chromatography analysis, Thomsonide contained 1.84% glycitein, 2.45% tectorigenin, 11.58% tectoridin, and 12.51% tectorigenin 7-*o*-xylosylglucoside as isoflavonoids, and 21.34% kakkasaponins as triterpenoid saponins.

2.2. Animals

Male ddY strain mice weighing 30–38 g were obtained from Japan SLC Co., Ltd. (Shizuoka, Japan). All animals were placed in cages and maintained in an air-conditioned room with illumination from 7 a.m. to 7 p.m. The room temperature ($22 \pm 2\,^{\circ}C$) and humidity ($55 \pm 10\%$) were controlled automatically. The animals were given access to laboratory chow (pellets) (Funabashi Nojo KK, Chiba, Japan) and water ad libitum.

2.3. Acute toxicity

Thomsonide suspension in 1% sodium carboxyl methyl cellulose (CMC-Na) at doses of 500 mg/kg and 5 g/kg, as a maximal practicable dose, was administered orally to mice in a volume of 0.1 ml per 10 g body weight and the animals were checked for any change in behavior during the 60-min period immediately after administration, according to the method described by Irwin (1964). In addition, the number of mice that died within 72 h after Thomsonide administration was counted.

2.4. Passive avoidance task

The passive avoidance test was performed according to the step-through method described by Zhang et al. (1994). The apparatus consists of a two-compartment acrylic box in which a lighted compartment is connected to a dark compartment by an entrance hole. Briefly, in the acquisition trial a mouse was placed in the lighted chamber, and when the mouse entered the dark chamber, a 0.4 mA electrical shock was given through floor grids until the mouse ran back into the lighted compartment. The latency times until the mice entered the dark compartment were measured with a stopwatch. A retention trial was performed 24 h after the acquisition trial, and latency times to reenter the dark chamber were measured. The success rate was then calculated as the number of mice that did not enter the dark compartment divided by the total number of mice and expressed as a percentage (%). The maximum entry latency time allowed in the acquisition session and retention session was 180 and 300 s, respectively. To impair memory registration, a dose of 30% ethanol (3 g/kg p.o.) or scopolamine hydrobromide (Tokyo Kasei Kogyo Co., Ltd, Tokyo, Japan) in saline (0.5 mg/kg i.p.) was given in a volume of 0.1 ml per 10 g of body weight 20 min before the acquisition trial. To impair memory retrieval, a dose of 40% ethanol (4 g/kg p.o.) was given 20 min before the retention trial. The 125, 250 and 500 mg/kg doses of Thomsonide suspension in 1% CMC-Na were administered orally in a volume of 0.1 ml per 10 g of body weight to mice 10 min before administration of ethanol or scopolamine hydrobromide. The control group was given the same volume of 1% CMC-Na. The normal group was given the same volume of vehicles instead of Thomsonide and ethanol or scopolamine hydrobromide.

2.5. Statistical analysis

Latency times are expressed as mean ± standard error of the mean (S.E.M.). The significance of differences was evaluated by one-way analysis of variance (ANOVA) and Dunnet's multiple range test, or the chi-square test.

3. Results

3.1. Acute toxicity

At the 500 mg/kg dose Thomsonide did not induce any marked changes in behavior, but transient depression of locomotor activity was observed for 30 min immediately after Thomsonide administration of the 5 g/kg dose. Even at the maximal practicable dose Thomsonide did not induce any marked change in behavior, except for the transient locomotor depression. Thomsonide did not cause any deaths within 72 h at either the 500 mg/kg or 5 g/kg dose.

3.2. Effect of Thomsonide on ethanol-induced impairment of memory registration in a passive avoidance task in mice

As shown in Table 1, the latency time to enter the dark chamber in the acquisition trial was significantly shorter in the mice given ethanol, i.e., the control group, as compared to the mice given the vehicle. However, there was no difference between the control group and the Thomsonide-treated group. The step-through latency time in the ethanol group in the retention trial was significantly shorter than in the vehicle group. Within the 125 to 500 mg/kg dose range pretreatment with Thomsonide dose-dependently increased the latency time and at the 500 mg/kg dose the difference was significant, as compared to the control group. The success rate in the vehicle group and control group was 92.86 and 0%, respectively. The differences in success rate between the control group and each of the Thomsonide-treated groups were not significant. However, the Thomsonide-treated mice exhibited confused behavior, such as hesitatingly wandering about the entrance hole connecting to the dark compartment.

Table 1
Effect of Thomsonide on ethanol-induced impairment of memory registration and memory retrieval in a passive avoidance task in mice

	Latency (s)			
	N	Acquisition trial	Retention trial	Success rate (%)
Memory registration				
Normal	14	41.36 ± 10.95*	296.71 ± 3.29**	13/14 (92.86)***
Control	14	10.93 ± 4.22	34.57 ± 7.03	0/14 (0)
Thomsonide				
125 mg/kg	12	17.75 ± 4.49	61.25 ± 14.07	0/12 (0)
250 mg/kg	12	9.50 ± 2.09	75.08 ± 26.60	0/12 (0)
500 mg/kg	12	35.58 ± 14.31	95.75 ± 20.97**	0/12 (0)
Memory retrieval				
Normal	14	19.64 ± 3.76	295.50 ± 4.50**	13/14 (92.86)***
Control	11	19.91 ± 2.62	52.45 ± 10.97	0/11 (0)
Thomsonide				
125 mg/kg	14	21.43 ± 4.81	90.00 ± 22.09	1/14 (7.14)
250 mg/kg	14	17.93 ± 2.50	60.71 ± 22.62	1/14 (7.14)
500 mg/kg	15	27.73 ± 5.41	139.53 ± 19.13**	1/15 (6.67)

*$p < 0.05$, **$p < 0.01$ (ANOVA and Dunnet's multiple range test) significantly different from control; ***$p < 0.01$ (chi-square test) significantly different from control.

3.3. Effect of Thomsonide on ethanol-induced impairment of memory retrieval in a passive avoidance task in mice

In the acquisition trial, there were no significant differences in latency time between the vehicle group, control group, and Thomsonide-treated group. In the retention trial, the latency time in the vehicle group and the control group was 295.50 ± 4.50 and 52.45 ± 10.97 s, respectively, and the latency time in the Thomsonide groups given the 125, 250, and 500 mg/kg doses was 90.00 ± 22.09, 60.71 ± 22.62, and 139.53 ± 19.13 s, respectively. The latency time in the control group was significantly shorter than in the vehicle group. At the 500 mg/kg dose Thomsonide significantly reversed the shorter latency induced by ethanol, but it was less effective at the 125 and 250 mg/kg doses. There were no significant differences in success rate between the control group and any of the Thomsonide-treated groups, but the success rate in the ethanol group, i.e., the control group, was significantly lower than in the vehicle group (Table 1). However, the Thomsonide-treated mice were observed to exhibit confused behavior, such as hesitatingly wandering about the entrance hole connected to the dark compartment. These results were similar to the results obtained in regard to memory registration impairment induced by ethanol.

3.4. Effect of Thomsonide on scopolamine-induced impairment of memory registration in a passive avoidance task in mice

In the acquisition trial, there were no significant differences in latency time between the vehicle group, scopolamine group, and Thomsonide-treated group. In the retention trial, the latency time in the vehicle group and scopolamine group was 292.46 ± 6.33 and 116.08 ± 23.66 s, respectively. The latency time in the Thomsonide groups given the 125, 250, and 500 mg/kg dose was 189.69 ± 27.69, 197.46 ± 24.63, and 253.00 ± 19.38 s, respectively. The latency time in the scopolamine group, i.e., the control group was significantly shorter than in the vehicle group. Within the 125 to 500 mg/kg dose range Thomsonide dose-dependently reversed the shorter latency induced by scopolamine, and at the 250 and 500 mg/kg doses its effect was significant, as compared to the control group. The success rate in the vehicle group and the scopolamine group was 84.62 and 8.33%, respectively, and the difference in success rate between the vehicle group and the control group was significant. The 500 mg/kg dose of Thomsonide significantly improved the scopolamine-induced reduction in success rate (Table 2). Thus, Thomsonide had an ameliorating effect on scopolamine-induced amnesia in relation to passive avoidance behavior.

4. Discussion

Since the shorter latency time in the ethanol group in the retention trial was reversed in the Thomsonide-treated group, the results of this study demonstrated that Thomsonide has an ameliorating effect on the impairment of both memory registration and memory retrieval induced by ethanol in regard to passive avoidance behavior. Its effects were adequate at the 500 mg/kg dose, which did not induce any marked change of behavior. These results indicate that Thomsonide has an antiamnesic effect on the central nervous system in alcoholic intoxication and support the traditional use of *Puerariae flos* for the treatment of alcoholic intoxication. Our findings also show that Thomsonide improved scopolamine-induced impairment of memory registration in passive avoidance behavior in mice. It is well known that the cholinergic system plays an important role in learning and memory (Kameyama

Table 2
Effect of Thomsonide on scopolamine-induced impairment of memory registration in a passive avoidance task in mice

	Latency (s)			
	N	Acquisition trial	Retention trial	Success rate (%)
Normal	13	19.31 ± 2.62	292.46 ± 6.33**	11/13 (84.62)****
Control	12	33.25 ± 5.81	116.08 ± 23.66	1/12 (8.33)
Thomsonide				
125 mg/kg	13	41.92 ± 8.44	189.69 ± 27.69	3/13 (23.08)
250 mg/kg	13	49.85 ± 7.38	197.46 ± 24.63*	4/13 (30.77)
500 mg/kg	12	56.75 ± 8.12	253.00 ± 19.38**	6/12 (50.00)***

*$p < 0.05$, **$p < 0.01$ (ANOVA and Dunnet' s multiple range test) significantly different from control; ***$p < 0.05$, ****$p < 0.01$ (chi-square test) significantly different from control.

et al., 1986), and there is evidence from both animal and human studies indicating that learning and memory can be modified by drugs that affect central cholinergic function (Bartus et al., 1982). Acute ethanol consumption has been reported to decrease cortical acetylcholine release (Morgan and Phillis, 1975) and turnover (Rawat, 1974), and Nabeshima et al. (1991) have reported that ethanol-induced amnesia is partially attenuated by both pre- and post-training administration of physostigmine, an acetylcholinesterase inhibitor. Therefore, the ethanol-induced impairment of learning and memory may at least in part be the result of reduced function of the cholinergic neuronal system. In light of these findings, our results suggest that the antiamnesic effect of Thomsonide on scopolamine-induced impairment of learning and memory may be related to modification of cholinergic neuronal systems. *Puerariae flos* is known to be a rich source of isoflavonoids and triterpenoid saponins, and isoflavonoids and triterpenoid saponins have been shown to have an ameliorating effect on memory. Isoflavonoid phytoestrogens have weak agonist activity at estrogen receptors, and Packard (1998) has reported that estrogen selectively influences memory storage independent of its effect on non-mnemonic processes, and that estrogen interacts with cholinergic systems in memory modulation. In view of the above evidence that isoflavonoid phytoestrogens may enhance memory through cholinergic neuronal functions, it is not surprising that Thomsonide, which is rich in isoflavonoids, such as tectridin, has an ameliorating effect on impairment of learning and memory. Thus, since our results suggest that the isoflavonoids contained in Thomsonide may improve the memory impairment induced by ethanol or scopolamine, isoflavonoids may enhance cholinergic neuronal functions through estrogen receptors. On the other hand, one of the saponin components of ginseng root, Rb1, which increases cholinergic function, has been shown to be effective in reversing memory and learning deficits in laboratory animals (Benishin et al., 1991), and Saito et al. (1977) have reported that Rb1 potentiates the effects of nerve growth factor. Based on all of these findings regarding the effects of saponin taken together, it is suspected that kakkasaponin, which is contained in Thomsonide, may improve the learning and memory impairment related to passive avoidance behavior in mice, in addition to the isoflavonoids. The results of the present study indicate that Thomsonide has an antiamnesic effect on the central nervous system in alcoholic intoxication and support the hypothesis that *Puerariae flos* may be useful in counteracting the effects of alcohol consumption. Since Thomsonide improved the scopolamine-induced impairment of memory registration related to passive avoidance, and scopolamine-induced amnesia is widely used as a model of senile dementia of the Alzheimer-type and of other learning and memory disorders, these results also suggest that *Puerariae flos* may provide the basis for a clinical strategy for the treatment of age-related memory impairment and dementia. Isolation of the effective components of Thomsonide and clarification of their pharmacological mechanisms is needed to confirm the findings in this study.

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