ELSEVIER

European Journal of Pharmacology 368 (1999) 237–243

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Effects of escins Ia, Ib, IIa, and IIb from horse chestnuts on gastric emptying in mice

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Received 6 August 1998; revised 11 January 1999; accepted 15 January 1999

Abstract

Inhibitory effects of the saponin fraction and its principal constituents, escins Ia, Ib, IIa, and IIb, from horse chestnuts on gastric emptying were investigated in mice loaded with a non-nutrient or nutrient meal. The saponin fraction and escins Ia–IIb inhibited gastric emptying of a 1.5% carboxymethyl cellulose sodium salt (CMC-Na) meal by 11.1–54.2% (12.5–200 mg/kg). Escins Ia–IIb (50 mg/kg) also inhibited gastric emptying of a 40% glucose meal by 21.1–23.5% except for escin Ia, a milk meal by 18.4–33.1%, and a 30% ethanol meal by 13.5–15.9%. The effects of escins Ia–IIb on gastric emptying of the CMC-Na meal were attenuated by pretreatment with streptozotocin (100 mg/kg, i.v.), capsaicin (75 mg/kg in total, s.c.), or insulin (1 U/kg, s.c.). The effect of insulin was reduced by glucose (2 g/kg, i.v.) which can directly nourish the brain, but not by fructose (2 g/kg, i.v.) which cannot be utilized by the brain. The effects of escins Ia–IIb (50 mg/kg) were overridden in 60% ethanol-loaded mice, in which the central nervous system was suppressed by ethanol. These results suggest that capsaicin-sensitive sensory nerves and central nervous system partly participate in the effects of escins Ia–IIb.

Keywords: Gastric emptying; Escin; Capsaicin-sensitive sensory nerve; Central nervous system

1. Introduction

The saponin mixture ‘escin’ obtained from horse chestnuts, the seeds of *Aesculus hippocastanum* L., is widely used in the therapy of peripheral vascular disorders and in cosmetics for prevention and treatment of cellulitis. The isolation and structure elucidation of the saponin constituents in ‘escin’, however, remained to be done. We have recently isolated twelve acylated polyhydroxyolean-12-ene 3-*O*-monodesmosides, escins Ia, Ib, IIa, IIb, IIIa, IIIb, IV, V, and VI and isoescins Ia, Ib, and V, from horse chestnuts and determined their chemical structures (Yoshikawa et al., 1994, 1996, 1998). We have also reported on both the inhibitory activity of the principal saponins, escins Ia, Ib, IIa, and IIb (Fig. 1), on increased blood glucose or ethanol concentration in oral glucose- or ethanol-loaded rats (Yoshikawa et al., 1994, 1996), and their anti-inflammatory effects (Matsuda et al., 1997). Furthermore, investigation of the modes of action of escins Ia and IIa for the hypoglycemic activity revealed that escins Ia and IIa strongly inhibited gastric emptying in rats and that they also inhibited glucose uptake in the small intestine in vitro (Matsuda et al., 1998). We now describe the inhibitory effects of the saponin fraction and escins Ia–IIb on gastric emptying in non-nutrient meal- or nutrient meal-loaded mice. We also discuss the roles of capsaicin-sensitive sensory nerves, central nervous system, and sympathetic nervous system in these effects.

2. Materials and methods

2.1. Chemicals

The saponin fraction and escins Ia, Ib, IIa, and IIb were isolated from the seeds of *Aesculus hippocastanum* using our method reported upon previously (Yoshikawa et al., 1996). Milk powder consisting of 13.0% protein, 27.8% lipids, and 54.2% carbohydrates was purchased from Snow Brand Milk, Japan. Other substances were purchased from Wako, Japan.

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PII: S0014-2999(99)00035-7

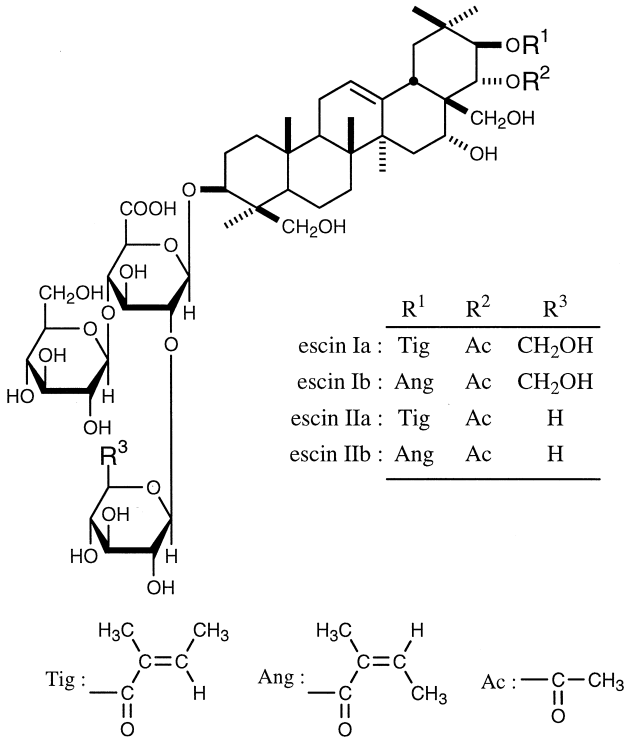


Fig. 1. Chemical structures of escins Ia, Ib, IIa, and IIb.

2.2. Animals

Male ddY mice, weighing 27–30 g, were purchased from Kiwa Laboratory Animal, Japan. The animals were maintained at a constant temperature of 23 ± 2°C and were fed a standard laboratory chow (MF, Oriental Yeast, Japan) for a week. The animals were fasted for 18–20 h prior to experiments, but were supplied with water ad libitum. Escins Ia–IIb were dissolved in phosphate-buffered saline. The solution was administered orally at 10 ml/kg in each experiment, while the vehicle was given orally at 10 ml/kg in the corresponding control group. The experiments were performed with conscious animals unless otherwise noted and were approved by Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.3. Measurement of gastric emptying

Gastric emptying was determined with a modification of the phenol red method (Barquist et al., 1996; Taché et al., 1987). A solution of 1.5% carboxymethyl cellulose sodium salt (CMC-Na), 40% glucose, milk [milk powder: water (w/w) = 1:3], or 60% ethanol containing 0.05% phenol red as a marker was given intragastrically (0.5 ml/mouse) to conscious mice. Thirty minutes later, the mice were killed by cervical dislocation. The abdominal cavity was opened, and the gastroesophageal junction and the pylorus were clamped, then the stomach was removed, weighed and placed in 14 ml of 0.1 N NaOH and homogenized. The suspension was allowed to settle for 1 h at room temperature and 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid (w/v) and then centrifuged at 3000 rpm for 20 min. The supernatant was mixed with 4 ml of 0.5 N NaOH, and the amount of phenol red was determined from the absorbance of the sample read at 560 nm (Beckman, DU530, Life Science UV/VIS, Spectrophotometer). Phenol red recovered from animals killed immediately after administration of the test meal was used as standard (0% emptying). Gastric emptying (%) in the 30-min period was calculated according to the following equation:

$$\text{gastric emptying (\%)} = (1 - \text{amount of test sample} / \text{amount of standard}) \times 100$$

The estimated dose of test compound yielding 30% inhibition (ID_{30}) was calculated from a graph of the dose–response curve.

2.4. Measurement of serum glucose

Blood samples were collected from the retro-orbital sinus just before the mice were killed in order to measure serum glucose levels. Serum glucose levels were determined with the glucose oxidase method (kit reagent: Glucose CII-test Wako, Wako).

2.5. Gastric emptying of test meals of 1.5% CMC-Na, 40% glucose, milk, 30 and 60% ethanol in normal mice

The fasted mice were placed in separate cages for 2 h. The test sample was given orally by means of a metal orogastric tube, and the test meal (0.5 ml/mouse) was administered orally 30 min later. The gastric emptying (%)

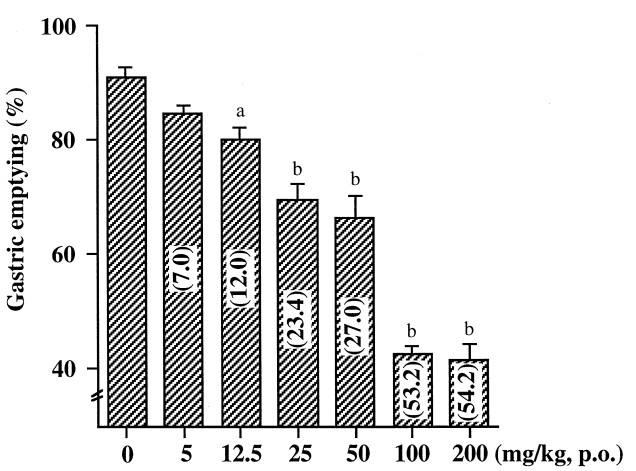


Fig. 2. Effects of the saponin fraction from horse chestnuts on gastric emptying in non-nutrient meal-loaded mice. Gastric emptying was determined 30 min after administration of a 1.5% CMC-Na test meal. The saponin fraction was given orally 30 min before administration of the test meal. Bars and values in parentheses represent the means with S.E.M. ($n = 8$) and the inhibition (%). Significantly different from the control group, $^{a}P < 0.05$, $^{b}P < 0.01$ (Dunnett's test).

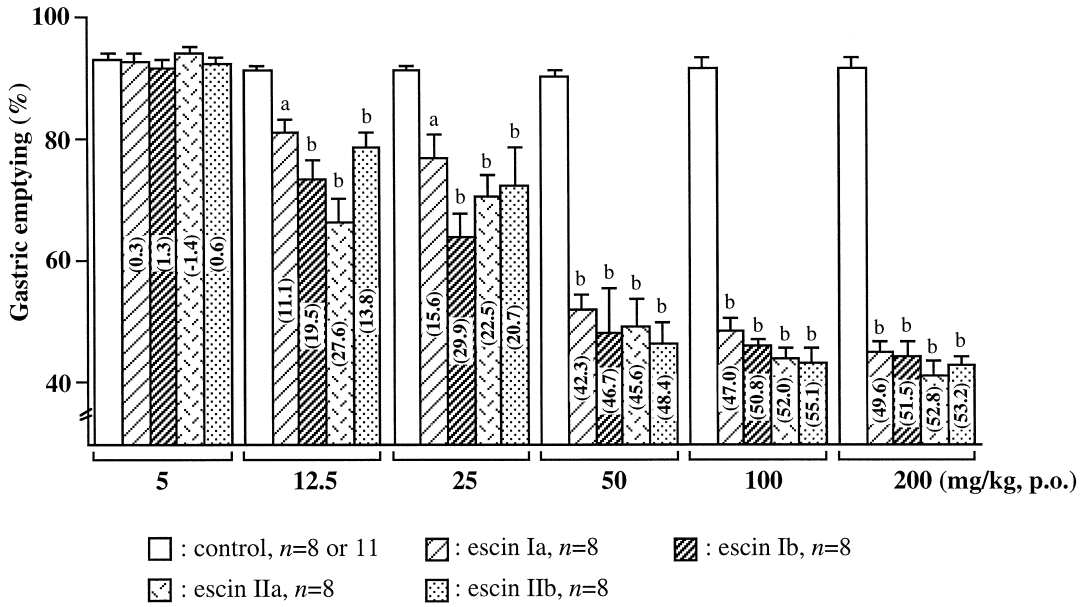


Fig. 3. Effects of escins Ia, Ib, IIa, and IIb on gastric emptying in non-nutrient meal-loaded mice. Gastric emptying was determined 30 min after administration of a 1.5% CMC-Na test meal. Each test compound (5–200 mg/kg, p.o.) was given 30 min before administration of the 1.5% CMC-Na test meal. Bars and values in parentheses represent the means with S.E.M. and the inhibition (%). Significantly different from the control group, $^{a}P < 0.05$, $^{b}P < 0.01$ (Dunnett's test).

and the serum glucose levels were determined 30 min after administration of the test meal.

2.6. Gastric emptying in streptozotocin-induced hyperglycemic mice

Streptozotocin (100 mg/kg, i.v.), dissolved in 10 ml citrate buffer (pH 4.2), was administered to the 20-h fasted mice 7 days before the administration of the sample. Mice with a serum glucose level above 250 mg/dl while fasted, considered to be diabetic, were used for the experiment. The gastric emptying (%) and the serum glucose levels were determined 30 min after administration of the test meal.

2.7. Gastric emptying in capsacin-pretreated mice

Capsaicin solution was prepared in a solution containing 99.5% ethanol, Tween 80, and saline (2: 1: 7, v/v/v). Mice (30–33 g) were injected with sodium pentobarbital

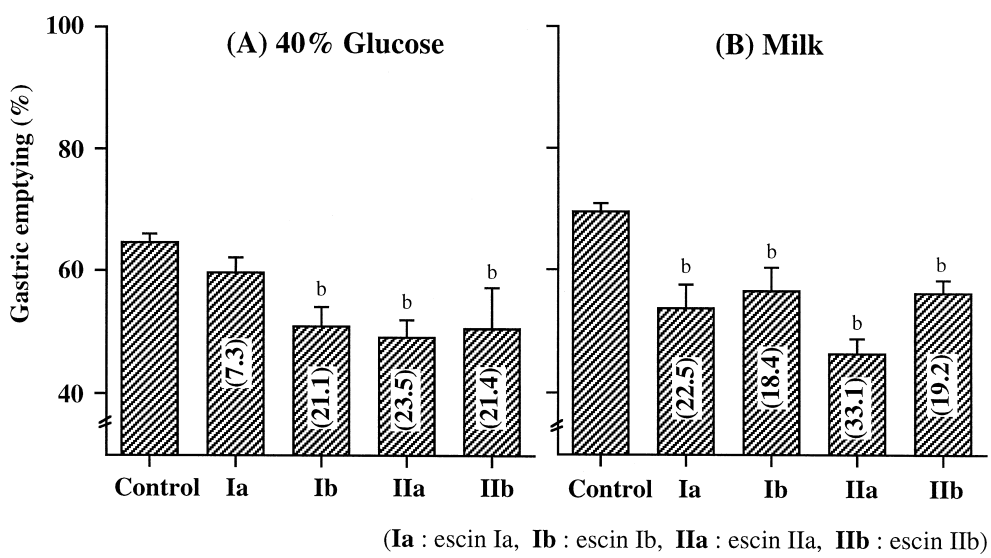
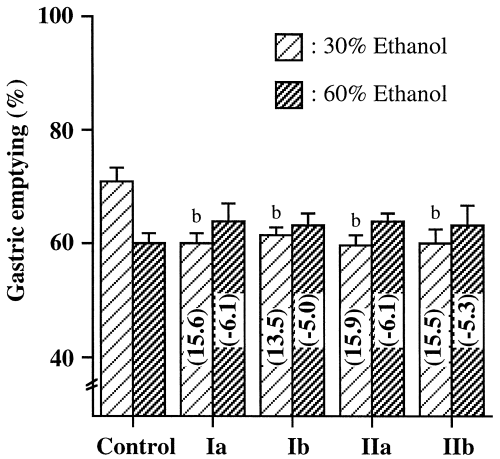


Fig. 4. Effects of escins Ia, Ib, IIa, and IIb on gastric emptying in nutrient meal-loaded mice. Gastric emptying was determined 30 min after administration of a 40% glucose or milk test meal. Each test compound (50 mg/kg, p.o.) was given 30 min before administration of the test meal. Bars and values in parentheses represent the means with S.E.M. ($n = 8$, 12) and the inhibition (%). Significantly different from the control group, $^{a}P < 0.05$, $^{b}P < 0.01$ (Dunnett's test).



(**Ia** : escin Ia, **Ib** : escin Ib, **IIa** : escin IIa, **IIb** : escin IIb)

Fig. 5. Effects of escins Ia, Ib, IIa, and IIb on gastric emptying in 30 or 60% ethanol test meal-loaded mice. Gastric emptying was determined 30 min after administration of a 30 or 60% ethanol test meal. Each test compound (50 mg/kg, p.o.) was given 30 min before administration of the test meal. Bars and values in parentheses represent the means with S.E.M. ($n = 8$) and the inhibition (%). Significantly different from the control group, $^{b}P < 0.01$ (Dunnett's test).

(30 mg/kg, i.p.) 15 min before, and were treated with increasing doses of capsaicin for two consecutive days (25 and 50 mg/kg, s.c.) to deplete neuropeptides in primary afferent neurons as a modification of the method described previously (Barrachina et al., 1997). To counteract any respiratory impairment associated with administration of capsaicin, the mice were pretreated with aminophylline (10 mg/kg, dissolved in 5 ml saline, i.m.) before capsaicin injection. After 14 days, the efficiency of capsaicin pretreatment was verified, using the corneal chemosensory test which consists of monitoring the wiping reflex in response to ocular instillation of a drop of 0.1% NH_4OH solution. None of the capsaicin-pretreated mice showed a wiping response, indicating effective ablation of primary sensory afferents, whereas the wiping reflex was present in vehicle-pretreated mice. Gastric emptying (%) was evaluated 30 min after administration of the test meal.

2.8. Gastric emptying in insulin-induced hypoglycemic mice

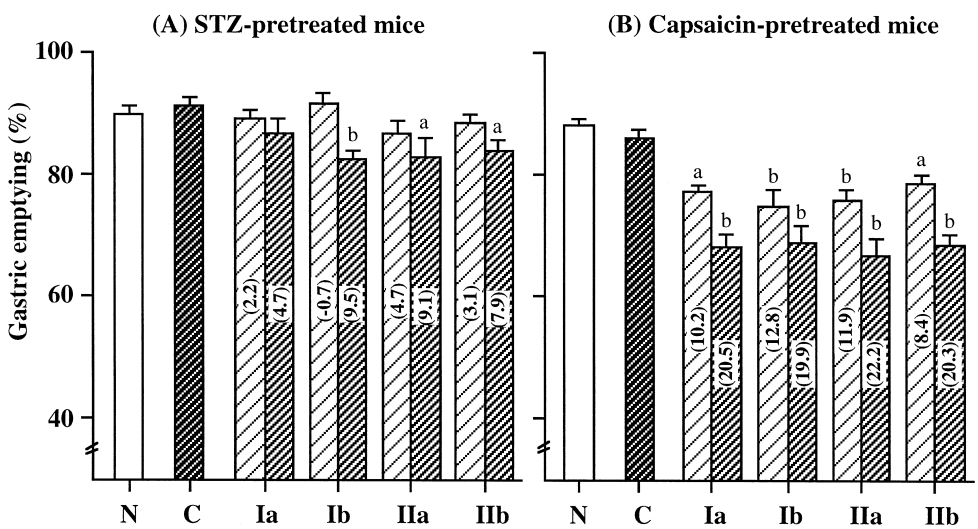
Insulin (1 U/kg, dissolved in 0.1 N HCl and diluted in saline, s.c.) was administered to the fasted mice 30 min before administration of the samples. The gastric emptying (%) and the serum glucose levels were determined 30 min after administration of the test meal.

2.9. Gastric emptying in mice pretreated with insulin in combination with glucose or fructose

Insulin (1 U/kg, s.c.) and glucose (2 g/kg, i.v.) or fructose (2 g/kg, dissolved in saline, i.v.) were injected in fasted mice 30 min before the administration of the sample. Gastric emptying (%) was evaluated 30 min after administration of the test meal.

2.10. Statistics

Values were expressed as means ± S.E.M. One-way analysis of variance following Dunnett's test or Scheffé's



(**N** : normal, **C** : control, **Ia** : escin Ia, **Ib** : escin Ib, **IIa** : escin IIa, **IIb** : escin IIb, ▨ : 25 mg/kg, ▨ : 50 mg/kg)

Fig. 6. Effects of escins Ia, Ib, IIa, and IIb on gastric emptying in STZ- or capsaicin-pretreated mice. Mice were pretreated with STZ (100 mg/kg, i.v.) or capsaicin (75 mg/kg in total, s.c.). Seven days or fourteen days thereafter, a 1.5% CMC-Na test meal was administered to the 20-h fasted mice. Gastric emptying was determined 30 min after the administration of the test meal. Each test compound (25 and 50 mg/kg, p.o.) was given 30 min before the test meal. Bars and values in parentheses represent the means with S.E.M. ($n = 8$) and the inhibition (%). Significantly different from the control group, $^{a}P < 0.05$, $^{b}P < 0.01$ (Dunnett's test).

test for multiple comparisons was used for statistical analysis. Probability (P) values less than 0.05 were considered significant.

3. Results

3.1. Effects of the saponin fraction and escins Ia–IIb on gastric emptying in normal mice given a non-nutrient meal

The inhibitory effects of the saponin fraction and its principal saponins (escins Ia, Ib, IIa, and IIb) on 30-min gastric emptying of 1.5% CMC-Na in normal mice are summarized in Figs. 2 and 3.

The saponin fraction and escins Ia–IIb (12.5–200 mg/kg) inhibited the gastric emptying of the 1.5% CMC-Na meal by 11.1–54.2%, with a near to maximal effect at 100 mg/kg. Their ID_{30} values were estimated to be 40, 40, 23, 23, and 29 mg/kg, respectively. There were 30–50% of mice that manifested a toxic reaction with cold body, hemostasis and diarrhea at the dose of 200 mg/kg.

3.2. Effects of escins Ia–IIb on gastric emptying in normal mice given nutrient meal

Escins Ia–IIb (50 mg/kg) inhibited the gastric emptying of a 40% glucose meal by 21.1–23.5% except for escin Ia (Fig. 4A) and of a milk meal by 18.4–33.1% (Fig. 4B). Escins Ia–IIb, except for escin Ia, tended to decrease the serum glucose levels in the 40% glucose test meal-loaded mice (control: 400.4 ± 25.4 mg/dl, escin Ia: 411.0 ± 18.3 mg/dl, escin Ib: 383.6 ± 25.9 mg/dl, escin IIa: 336.4 ± 9.7 mg/dl, escin IIb: 326.3 ± 24.2 mg/dl).

3.3. Effects of escins Ia–IIb on gastric emptying in normal mice given a 30 or 60% ethanol meal

Escins Ia–IIb (50 mg/kg) inhibited the gastric emptying of a 30% ethanol meal by 13.5–15.6% (Fig. 5). There was, however, no such effect in 60% ethanol meal-loaded mice, in which the central nervous system was suppressed by the large dose of ethanol.

3.4. Effects of escins Ia–IIb on gastric emptying in streptozotocin-pretreated mice given a non-nutrient meal

As shown in Fig. 6A, pretreatment with streptozotocin (100 mg/kg, i.v.) increased the levels of serum glucose about fourfold [control: 360.1 ± 19.1, normal: 99.5 ± 5.5 ($P < 0.01$)]. The pretreatment with streptozotocin almost abolished the effects of escins Ia–IIb (25 and 50 mg/kg).

3.5. Effects of escins Ia–IIb on gastric emptying in capsaicin-pretreated mice given a non-nutrient meal

As shown in Fig. 6B, pretreatment with capsacin (75 mg/kg in total) did not influence the rate of gastric emptying. This pretreatment attenuated the inhibitory effects of escins Ia–IIb (25 and 50 mg/kg) on gastric emptying (%).

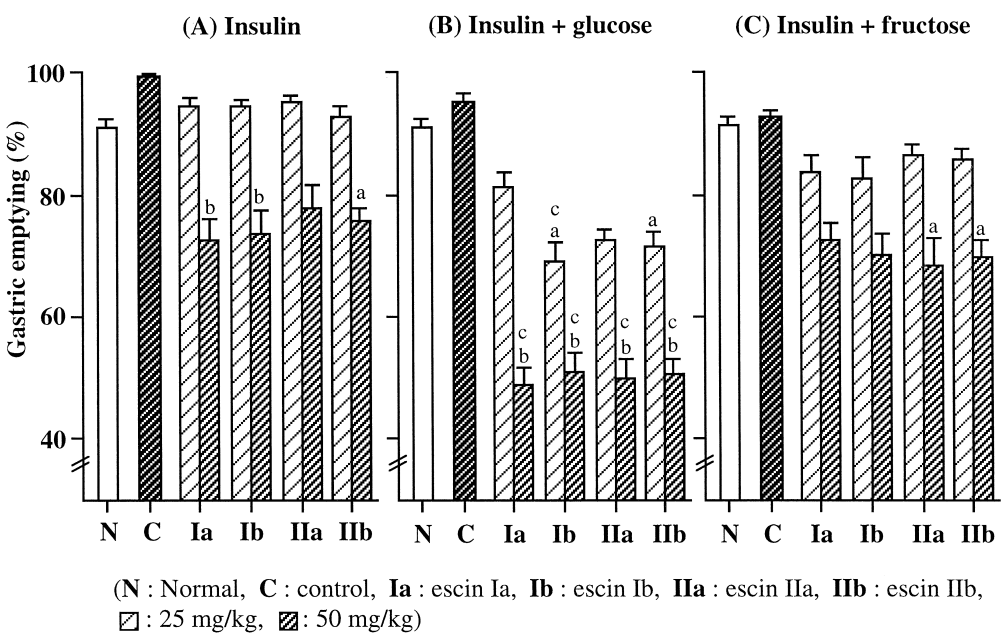


Fig. 7. Effects of escins Ia, Ib, IIa, and IIb on gastric emptying in insulin-, insulin and glucose- or insulin and fructose-pretreated mice. Insulin (1 U/kg, s.c.) and glucose (2 g/kg, i.v.) or fructose (2 g/kg, i.v.) were injected 30 min before the administration of the sample. Gastric emptying was determined 30 min after administration of a CMC-Na test meal. Each test compound (25 and 50 mg/kg, p.o.) was given 30 min before the test meal. Bars represent the means with S.E.M. ($n = 8–10$). [a,b]Significantly different from the corresponding control group in each panel, [a]$P < 0.05$, [b]$P < 0.01$. [c]Significantly different from the corresponding insulin-pretreated group in panel A, [c]$P < 0.05$ (Scheffé's test).

3.6. Effects of escins Ia–IIb on gastric emptying in insulin-, insulin and glucose- or insulin and fructose-pretreated mice given a non-nutrient meal

Pretreatment with insulin (1 U/kg, s.c.) tended to increase the gastric emptying (%) and markedly decreased the serum glucose levels [control: 35.3 ± 3.3 mg/dl, normal: 104.3 ± 4.2 mg/dl ($P < 0.01$)]. This pretreatment markedly attenuated the inhibitory effects of escins Ia–IIb (25 and 50 mg/kg) on gastric emptying (Fig. 7A). In comparison with the effects of pretreatment with insulin (1 U/kg, s.c.) alone, pretreatment with a combination of insulin and glucose (2 g/kg, i.v.) slightly influenced the rate of gastric emptying, but apparently reversed the effects of escins Ia–IIb on gastric emptying at doses of 25 and/or 50 mg/kg (Fig. 7B). On the other hand, pretreatment with a combination of insulin and fructose (2 g/kg, i.v.) did not reverse the effects of escins Ia–IIb (Fig. 7C).

4. Discussion

The present results demonstrated that the saponin fraction from horse chestnuts and its principal saponins, escins Ia–IIb (12.5–200 mg/kg), dose dependently inhibited gastric emptying in mice loaded with a non-nutrient meal (1.5% CMC-Na test meal). Escins Ia–IIb (50 mg/kg) also inhibited gastric emptying in mice given the nutrient test meals (40% glucose test meal and milk test meal) and a 30% ethanol test meal, except for escin Ia with the 40% glucose test meal-loaded mice. Among these saponins, escins Ib, IIa, and IIb seemed to be equipotent, while escin Ia had the least effect on inhibition of gastric emptying in mice loaded with the 1.5% CMC-Na test meal and 40% glucose test meal.

The speed of gastric emptying is important in the regulation of glucose homeostasis (Horowitz et al., 1993). Gastric emptying abnormalities are common in diabetic patients and animals (Kong et al., 1996). It was reported that gastric emptying is faster in type II diabetic patients (Phillips et al., 1992), type I diabetic patients (Nowak et al., 1990; Pehling et al., 1984), and diabetic rodents (Chang et al., 1996; Green et al., 1997; Nowak et al., 1994) as compared to healthy controls. Some studies have shown that obese subjects had accelerated gastric emptying as compared to that in healthy controls (Tosetti et al., 1996). Treatment with insulin and other hypoglycemic agents can increase gastric emptying in patients and animals with diabetes mellitus. More rapid gastric emptying rates in patients with diabetes mellitus would result in more rapid absorption of food, and therefore higher postprandial glucose levels. Consequently, slowing gastric emptying will prolong the postprandial absorption of food, with a resultant improvement in blood glucose control. It should be investigated whether the inhibition of gastric emptying induced by the saponin fraction and its principal saponins, escins Ia–IIb, is of benefit for the prevention and treatment of diabetes and morbid obesity with accelerated gastric emptying.

Capsaicin is widely used to ablate sensory C fibers. It has been used systemically to ablate all capsaicin-sensitive C fibers. The inhibition of gastric emptying by some hormones, such as secretin and cholecystokinin, is mediated via a capsaicin-sensitive vagal afferent pathway (Raybould and Holzer, 1993; Raybould et al., 1994). The effects of escins Ia–IIb on gastric emptying were markedly attenuated in the mice systemically pretreated with capsaicin. It is not clear whether escins Ia–IIb stimulate the release of such hormones. This finding, however, suggests the inhibition of gastric emptying by escins Ia–IIb is, at least in part, mediated via capsaicin-sensitive sensory nerves.

Gastric emptying was increased in insulin-induced hypoglycemia (McCann and Stricker, 1986). A central signal, perhaps originating in the cerebral chemoreceptors involved in mediating the responses (Flatt et al., 1974; Stricker et al., 1977), initiated the increased vagal activity to the stomach that resulted in increased gastric emptying (Bacharch, 1953). In the present study, gastric emptying in mice tended to be accelerated by insulin and the inhibition of gastric emptying induced by escins Ia–IIb was attenuated by the insulin-induced hypoglycemia. The effects of insulin-induced hypoglycemia on the inhibition of gastric emptying by escins Ia–IIb were markedly abolished by the intravenous injection of glucose, a sugar that can directly nourish the brain, but not by the intravenous injection of fructose, which cannot cross the blood-brain barrier to be utilized by the brain but is used readily by peripheral tissues (Oldendorf, 1971). Furthermore, the effects of escins Ia–IIb on gastric emptying were overridden in 60% ethanol-loaded mice, in which the central nervous system was suppressed. These results suggest that the mechanism of the inhibition of gastric emptying by escins Ia–IIb involves the central nervous system.

The sympathetic nervous system can play an important role in gastric emptying. For example, sympathetic activation may enhance the synthesis of prostaglandins to modulate gastric emptying (Kuratani et al., 1994; Stein et al., 1994). Hyperglycemia in strteptozotocin-induced hypoinsulinemic rats reduced the activity of the sympathetic nervous system (Young et al., 1983). In the present study, the inhibition of gastric emptying by escins Ia–IIb was markedly attenuated in streptozotocin-pretreated mice. Therefore, a mechanism involving the sympathetic nervous system should be considered for the inhibition of gastric emptying by escins Ia–IIb.

5. Conclusion

These results suggest that the capsaicin-sensitive sensory nerves and central nervous system partly participate

in the mechanism of the inhibitory activity of escins Ia–IIb on gastric emptying in mice. The mechanism of the sympathetic nervous system involvement need to be studied further.

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