Inhibition of Gastric Emptying by Triterpene Saponin, Momordin Ic, in Mice: Roles of Blood Glucose, Capsaicin-Sensitive Sensory Nerves, and Central Nervous System

H. MATSUDA, Y. LI, J. YAMAHARA, and M. YOSHIKAWA
Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto, Japan
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ABSTRACT

The roles of capsaicin-sensitive sensory nerves and the central nervous system in the inhibitory effect of momordin Ic, a principal saponin constituent in various Chinese and Japanese herbal medicines, such as the fruit of Kochia scoparia (L.) SCHRAD., on gastric emptying were investigated in nonnutrient meal- or nutrient meal-loaded mice. Momordin Ic (12.5—50 mg/kg) significantly inhibited gastric emptying in 1.5% carboxymethyl cellulose sodium salt test meal-loaded mice by 8.4%—60.6%, 40% glucose test meal-loaded mice by 42.8% (50 mg/kg), milk test meal-loaded mice by 36.4% (50 mg/kg), and 60% ethanol test meal-loaded mice by 37.2% (50 mg/kg). The inhibitory effect on the gastric emptying in 1.5% carboxymethyl cellulose sodium salt test meal-loaded mice was potentiated by glucose (2 g/kg, i.v. or 5 g/kg, i.p.), but markedly attenuated by pretreatment with alloxan (50 mg/kg, i.v.) and streptozotocin (100 mg/kg, i.v.), in which the activity of sympathetic nervous system was decreased, or by insulin (1 or 3 U/kg, s.c.). The effect of insulin (1 U/kg) was markedly 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 used by the brain.

In this report, we describe the inhibitory effects of momordin Ic (Fig. 1), which showed the stronger activity among these saponins, on the gastric emptying in nonnutrient meal- or nutrient meal-loaded mice. We also discuss the roles of blood glucose, capsaicin-sensitive sensory nerves, and the central nervous system in these effects in conscious mice.

In the course of our studies on bioactive saponin constituents in herbal medicines, we recently found that the extracts of several herbal medicines showed inhibitory activity on the increase of serum glucose level in oral glucose-loaded rats. Through bioassay-guided separation, we characterized their active saponin constituents, which could be classified into the following three types: 1) olean-12-en-28-oic acid 3-O-monodesmoside [Kochia scoparia (fruit), Aralia elata (roots, bark, and young shoots), and Beta vulgaris (roots and leaves)]; 2) acylated polyhydroxyolean-12-ene 3-O-monodesmoside [Aesculus hippocastanum (seeds) and Gymnema sylvestre (leaves)]; and 3) olean-12-ene 3,28-O-acylated bisdesmoside [Polygala senega var. latifolia (roots)] (Yoshikawa et al., 1995, 1996a,b,c,d, 1997a,b,c). Furthermore, investigation of the modes of action for the hypoglycemic activity of these types of constituents using representative saponins (momordin Ic, oleanolic acid 3-O-glucuronide, escins Ia and IIa, and senegin II) revealed that they inhibited gastric emptying in rats and also inhibited glucose uptake in the small intestine in vitro (Matsuda et al., 1998a,c). The dried fruit of Kochia scoparia, which is abundant in momordin Ic, has been orally administered for more than 2000 years in China for the treatment of diseases of the skin, urinary tract, and eyes and also used in Japan as a foodstuff. Momordin Ic was found to inhibit ethanol- and indomethacin-induced gastric mucosal lesions (Matsuda et al., 1998b). In this report, we describe the inhibitory effects of momordin Ic (Fig. 1), which showed the stronger activity among these saponins, on the gastric emptying in nonnutrient meal- or nutrient meal-loaded mice. We will also discuss the roles of blood glucose, capsaicin-sensitive sensory nerves, the central nervous system (CNS), and the sympathetic nervous system in these effects in conscious mice.

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In this report, we describe the inhibitory effects of momordin Ic (Fig. 1), which showed the stronger activity among these saponins, on the gastric emptying in nonnutrient meal- or nutrient meal-loaded mice. We also discuss the roles of blood glucose, capsaicin-sensitive sensory nerves, and the central nervous system in these effects in conscious mice.

Materials and Methods

Chemicals. Momordin Ic, colorless fine crystals; m.p. 240°C (dec.), was isolated and purified by HPLC from the dried fruit of Kochia scoparia L. (SCHRAD.) using the method reported previously (Yoshikawa et al., 1997c). Milk powder consisting of 13.0% protein, 27.8% lipids, and 54.2% carbohydrates was purchased from Snow Brand Milk Co. Ltd., Sapporo, Japan. Other reagents were purchased from Wako Pure Chemical Industries, Osaka, Japan.

Animals. Male ddY mice, weighing 27—30 g, were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were maintained at a constant temperature of 23 ± 2°C and were fed standard laboratory chow (MF, Oriental Yeast Co., Ltd.,
Tokyo, Japan) for a week. The animals were fasted for 18—20 h before experiments, but were supplied with water ad libitum. Momordin Ic was dissolved in PBS and was orally administered at 10 ml/kg in each experiment, whereas the vehicle was orally administered at 10 ml/kg in the corresponding control group. The experiments were performed in conscious animals unless otherwise noted.

**Measurement of Gastric Emptying.** Gastric emptying was determined by a modification of the phenol red method (Barquist et al., 1996; Tache et al., 1987). A solution of 1.5% carboxymethyl cellulose sodium salt (CMC-Na), 40% glucose, milk [milk powder: water (w/w) 5:1:3], or 60% ethanol containing 0.05% phenol red as a marker was given intragastrically (0.5 ml/mouse) to conscious mice. Thirty minutes later, mice were sacrificed 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 then 5 ml of the supernatant was added to 0.5 ml of 0.5% phenol red as a marker was given intragastrically (0.5 ml/mouse) to conscious mice. Thirty minutes later, mice were sacrificed 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 then 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid (w/v) and 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 at 560 nm (Beckman, DU530, Life Science UV/Vis, Spectrophotometer, Fullerton, CA). Phenol red recovered from animals that were sacrificed immediately after administration of the test meal was used as a standard (0% emptying).

Gastric emptying (%) in the 30-min period was calculated according to the following equation:

\[
\text{gastric emptying (%) = } \left(1 - \frac{\text{amount of test sample}}{\text{amount of standard}}\right) \times 100
\]

**Measurement of Serum Glucose.** Blood samples were collected from the retro-orbital sinus just before the mice were sacrificed to measure serum glucose levels. Serum glucose levels were determined by the glucose-oxidase method (kit reagent: Glucose CII-test Wako, Wako Pure Chemical Industries).

**Gastric Emptying of Test Meals of 1.5% CMC-Na, 40% Glucose, Milk, and 60% Ethanol in Normal Mice.** A 20% glucose saline solution (10 ml/kg, i.v.), 50% glucose saline solution (10 ml/kg, i.p.), or insulin (1 U/kg or 3 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 sample. The rate of gastric emptying and the serum glucose levels were determined 30 min after administration of the test meal.

**Gastric Emptying in Glucose (i.v. or i.p.)-Induced Hyperglycemic and Insulin-Induced Hypoglycemic Mice.** A 20% glucose saline solution (10 ml/kg, i.v.), 50% glucose saline solution (10 ml/kg, i.p.), or insulin (1 U/kg or 3 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 sample. The rate of gastric emptying and the serum glucose levels were determined 30 min after administration of the test meal.

**Gastric Emptying in Alloxan- or Streptozotocin (STZ)-Induced Hyperglycemic Mice.** Alloxan (50 mg/kg, dissolved in 10 ml saline solution) or STZ (100 mg/kg, dissolved in 10 ml citrate buffer, pH 4.2) was injected i.v. to the 20-h fasted mice 3 or 7 days before administration of the sample. Mice with a serum glucose level above 250 mg/dl, considered to be diabetic, were used in this study. The rate of gastric emptying and the serum glucose levels were determined 30 min after administration of the test meal.

**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 insulin (1 U/kg, s.c.) and fructose (2 g/kg, dissolved in saline, i.v.) were injected into the fasted mice 30 min before administration of the sample. The rate of gastric emptying and the serum glucose levels were determined 30 min after administration of the test meal.

**Gastric Emptying in Capsaicin-Pretreated Mice.** Capsaicin solution was prepared in a solution containing 99.5% ethanol, Tween

![](Fig.1.png)

**Fig. 1.** Chemical structure of momordin Ic

![](Fig.2.png)

**Fig. 2.** Effects of momordin Ic on gastric emptying and serum glucose levels in mice. Gastric emptying was determined 30 min after administration of test meal. Momordin Ic was given orally 30 min before administration of test meal. Bars represent means with S.E.M. of gastric emptying (%) and serum glucose level (mg/dl). Significantly different from control group, *p < .05, **p < .01.
80, and saline (2:1:7, v/v/v). Mice were anesthetized with sodium pentobarbital (30 mg/kg, i.p.), and treated with increasing doses of capsaicin for 2 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.) 30 min before capsaicin injection. After 14 days, the efficiency of capsaicin pretreatment was verified by the corneal chemosensory test that consists of monitoring the wiping reflex to ocular instillation of a drop of 0.1% NH₄OH solution. None of the capsaicin-pretreated mice showed a wiping response, indicating effective ablation of primary sensory afferents, whereas wiping reflex was present in vehicle-pretreated mice. The rate of gastric emptying and the serum glucose levels were determined 30 min after administration of the test meal.

Statistical Analyses. Values are expressed as means ± S.E.M. One-way ANOVA following Dunnett's test for multiple comparisons and Student's t test for a comparison of two groups were used for statistical analysis. Probability (p) values less than 0.05 were considered significant.

Results

The inhibitory effects of momordin Ic on gastric emptying for the different test meals in normal mice are shown in Fig. 2. The gastric emptying (%) at 30 min after loading with the test meal containing 1.5% CMC-Na, 40% glucose, milk, and 60% ethanol in normal mice were 92.2%, 57.2%, 67.8%, and 56.7%, respectively. Momordin Ic significantly inhibited gastric emptying in 1.5% CMC-Na test meal-loaded mice at doses of 12.5, 25, and 50 mg/kg by 8.4%, 23.5%, and 60.6%, respectively. It also inhibited gastric emptying in 40% glucose test meal-, milk test meal-, and 60% ethanol test meal-loaded mice at 50 mg/kg by 42.8%, 36.4%, and 37.2%, respectively. It significantly decreased the serum glucose levels in 40% glucose test meal- or milk test meal-loaded test meal-loaded mice.

As shown in Fig. 3, pretreatment with glucose (2 g/kg, i.v.) increased the effects of momordin Ic at the dose of 25 mg/kg on gastric emptying from 26.3% to 36.3% inhibition. The intraperitoneal injection of glucose (5 g/kg) markedly increased the levels of serum glucose by about 6-fold. The pretreatment potentiated the effects of momordin Ic at doses of 12.5, 25, and 50 mg/kg on the gastric emptying; inhibition was from 8.4% to 21.4%, from 23.5% to 37.3% and from 60.6 to 78.0%, respectively.

As shown in Fig. 4A, pretreatment with alloxan (50 mg/kg, i.v.) markedly increased the levels of serum glucose by about 6-fold. This pretreatment completely abolished the effects of momordin Ic at doses of 12.5 and/or 50 mg/kg, and attenuated the effects of momordin Ic at a dose of 50 mg/kg; inhibition was from 60.6% to 50.6% (1.5% CMC-Na test meal) and from 42.8% to 17.2% (40% glucose test meal), respectively. Pretreatment with a combination of alloxan and insulin completely abolished the effects of momordin Ic (25 and 50 mg/kg).
kg) on gastric emptying. Pretreatment with STZ (100 mg/kg, i.v.) markedly attenuated the effects of momordin Ic (25 and 50 mg/kg) on the rate of gastric emptying in 1.5% CMC-Na test meal- and 40% glucose test meal-loaded mice to 1.6% and 33.9%, and 1.2% and 16.9%, respectively, similarly to those of alloxan-induced diabetic mice (Fig. 4B).

As shown in Fig. 5, pretreatment with insulin (1 and 3 U/kg, s.c.) tended to increase gastric emptying and significantly decreased the serum glucose levels. This pretreatment markedly attenuated the effects of momordin Ic (25 and 50 mg/kg) on gastric emptying; inhibition was from 23.5% and 60.6% to 4.3% and 48.7%, and to 3.5% and 8.2%, respectively. The injection of glucose (2 g/kg, i.v.) reversed the effects of momordin Ic (25 and 50 mg/kg) on gastric emptying in insulin-pretreated mice, but the injection of fructose (2 g/kg, i.v.) did not.

As shown in Fig. 6, pretreatment with capsaicin (75 mg/kg in total, s.c.) attenuated the effects of momordin Ic (25 and 50 mg/kg) on gastric emptying; inhibition was from 23.5% and 60.6% to 8.7% and 40.0%, respectively.

**Discussion**

We reported that momordin Ic inhibited the gastric emptying in 10% glucose-loaded rats (Matsuda et al., 1998a), but its effects on the gastric emptying in mice after loading of nonnutrient meal, nutrient meal, and ethanol test meal and its mechanisms were unclarified. The present study demonstrated that momordin Ic (12.5—50 mg/kg) dose dependently inhibited gastric emptying in nonnutrient meal (1.5% CMC-Na test meal)-loaded mice, and also inhibited gastric emptying in animals given the nutrient meals (40% glucose test meal, milk test meal) and 60% ethanol test meal at the dose of 50 mg/kg. In addition, momordin Ic also significantly inhibited gastric emptying in mice with hyperglycemia induced by injection of glucose and with hypoglycemia induced by insulin, as well as in the alloxan- or STZ-induced diabetic mice. These results suggest that momordin Ic inhibits gastric emptying not only in normal mice but also in hyperglycemic (including diabetic) and hypoglycemic mice, and also not only in nonnutrient meal-loaded mice but also in nutrient meal-loaded mice.

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). The usual situation in patients with diabetes is delayed gastric emptying. But it was reported that gastric emptying occurred faster in some type 2 diabetic patients (Phillips et al., 1992), type 1 diabetic patients (Pehling et al., 1984; Nowak et al., 1990), and diabetic rodents (Nowak et al., 1994; Chang et al., 1996; Green et al., 1997) compared with healthy controls. Some studies have shown that obese subjects had accelerated gastric emptying compared with 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 of gastric emptying will prolong the postprandial absorption of food, with a resultant improvement in blood glucose control. Therefore, the inhibition of

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**Fig. 5.** Effects of momordin Ic on gastric emptying and serum glucose levels 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 into fasted mice 30 min before administration of momordin Ic. Gastric emptying and serum glucose levels were determined 30 min after administration of 1.5% CMC-Na test meal. Momordin Ic was given orally 30 min before administration of test meal. Bars represent means with S.E.M. of gastric emptying (%) and serum glucose level (mg/dl). Significantly different from control group, **p < .01.
by i.v. injection of glucose, a sugar that can directly nourish the brain, but not by the i.v. injection of fructose, a sugar that cannot cross the blood-brain barrier to be used by the brain (Oldendorff, 1971) and yet is used readily by peripheral tissues. These results suggest that the CNS participates in the inhibition of gastric emptying by momordin Ic.

Capsaicin has been used systematically to ablate all capsaicin-sensitive C fibers to produce a sensory pathway-specific ablation in rats (Holzer 1991; Zittel et al., 1994), and in mice (Barrachina et al., 1997). The inhibitions of gastric emptying by some hormones, such as secretin and cholecystokinin, are mediated via a capsaicin-sensitive vagal afferent pathway (Raybould and Holzer, 1993; Raybould et al., 1994). The effect of momordin Ic on gastric emptying was markedly attenuated by the pretreatment with capsicain. It is unclarified whether momordin Ic stimulates the release of such hormones, but this result suggests that capsaicin-sensitive sensory nerves are, at least in part, involved in the inhibition of gastric emptying by momordin Ic.

In conclusion, the present results suggest that the capsaicin-sensitive sensory nerves and CNS participate in the inhibitory activity of momordin Ic on gastric emptying, and the level of serum glucose can influence the effect of momordin Ic in normal mice. The mechanism of sympathetic nervous system need to be further studied.

References
Raybould HE, Zettel TT, Holzer HH, Lloyd KCR and Meyer JH (1994) Gastroodu-


