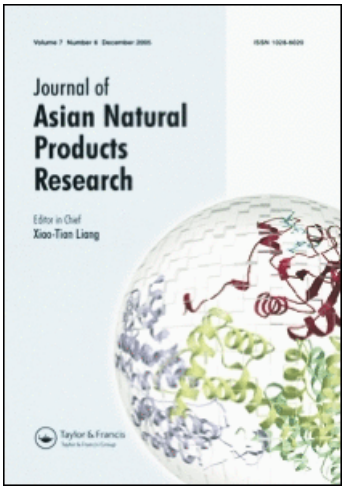

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Antihyperglycemic Effects of Gymnemic Acid IV, a Compound Derived from *Gymnema sylvestre* Leaves in Streptozotocin-Diabetic Mice

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ANTIHYPERGLYCEMIC EFFECTS OF GYMNEMIC ACID IV, A COMPOUND DERIVED FROM *GYMNEMA SYLVESTRE* LEAVES IN STREPTOZOTOCIN-DIABETIC MICE

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We investigated the antihyperglycemic action of a crude saponin fraction and five triterpene glycosides (gymnemic acids I–IV and gymnemasaponin V) derived from the methanol extract of leaves of *Gymnema sylvestre* R. Br. (Asclepiadaceae) in streptozotocin (STZ)-diabetic mice. The saponin fraction (60 mg/kg) reduced blood glucose levels 2–4 h after the intraperitoneal administration. Gymnemic acid IV, not the other 4 glycosides at doses of 3.4–13.4 mg/kg reduced the blood glucose levels by 13.5–60.0% 6 h after the administration comparable to the potency of glibenclamide, and did not change the blood glucose levels of normal mice. Gymnemic acid IV at 13.4 mg/kg increased plasma insulin levels in STZ-diabetic mice. Gymnemic acid IV (1 mg/mL) did not inhibit α-glycosidase activity in the brush border membrane vesicles of normal rat small intestines. These results indicate that insulin-releasing action of gymnemic acid IV may contribute to the antihyperglycemic effect by the leaves of *G. sylvestre*. Gymnemic acid IV may be an anti-obese and antihyperglycemic pro-drug.

Keywords: Gymnemic acid IV; *Gymnema sylvestre* R. Br. leaves; Blood-glucose lowering; Insulin release; Streptozotocin-diabetic mice

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INTRODUCTION

The leaves of *Gymnema sylvestre* R. BR. (Asclepiadaceae) is called "Gru-ma" an Indian word which means sugar-destroying. The crude extracts are used as medicinal foodstuffs and food additives in Japan. Ayurvedic herbal formulation including the crude drug has a strong antihyperglycemic activity on type 2 diabetic patients, and been used clinically for long-term without serious side effects in India [1]. The dried leaf powder or water-soluble extracts elicit the antihyperglycemic effect by recovering serum insulin levels to normal fasting levels in type 2 diabetic patients [2,3] and by regenerating the endocrine pancreas in diabetic rats [4]. Seventeen kinds of oleanane-type triterpenoid glycosides having antisweet activity have been isolated from the fresh root [5]. The crude saponin fraction from leaves classically named "gymnemic acid" suppresses sweet taste sensation [6,7]. Five triterpene glycosides (gymnemic acids I–IV and gymnemasaponin V) are isolated from the methanol extract and identified [8,9] (Fig. 1). Gymnemic acid I–IV have found to completely suppress sweet sensation [8]. Gymnemic acid II and IV at 0.5 mM inhibit significantly the glucose uptake into small intestinal fragments of normal rat [9]. In the present study we investigated the antihyperglycemic action of above five triterpene glycosides, the increasing effects on blood insulin levels in streptozotocin (STZ)-diabetic mice and the inhibiting effects on intestinal α-glycosidase activity in normal mice.

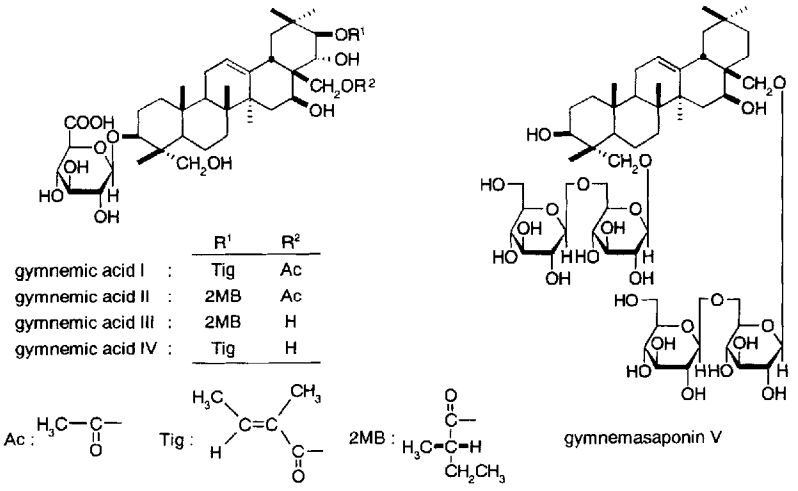

FIGURE 1 Chemical structures of gymnemic acid I, II, III and IV, and gymnemasaponin V.

RESULTS AND DISCUSSION

The crude saponin fraction from methanol extracts of the dried leaves of *G. sylvestre* was administered at 60 mg/kg into STZ-diabetic mice. The blood glucose levels were weakly lowered 2–4 h, and tended to be lowered 6 h after the injection (Fig. 2, upper). The antihyperglycemic effects of gymnemic acid IV at 3.4 13.4 mg/kg were in a dose-dependent manner by 13.5–60.0%, and at 13.4 mg/kg markedly lowered the blood glucose levels comparable to the activity of glibenclamide (14.8 mg/kg) 6 h after the application (Fig. 2, lower). But gymnemic acid IV did not change the blood glucose level of normal mice. Gymnemic acid IV at 13.4 mg/kg increased the plasma insulin level of STZ-diabetic mice (Fig. 3). Gymnemic acid IV (1 mg/mL) did not inhibit α-glycosidase (sucrase and maltase) activity in the brush border membrane vesicles of the normal rat small intestines. On the other hand, acarbose (2 μg/mL) inhibit α-glycosidase activity (sucrase 64.5% and maltase 51.5%). Gymnemic acids I, II, III and gymnemasaponin V (20 mg/kg, i.p.) did not affect the blood glucose level (data not shown).

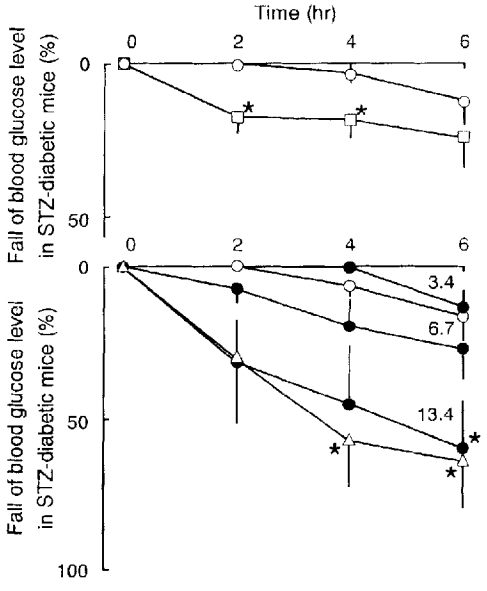

FIGURE 2 Time-dependent blood-glucose lowering by crude fraction of gymnemasaponins (□, 60 mg/kg, i.p., $n = 5$) (upper), and by gymnemic acid IV (●, 3.4, 6.7 and 13.4 mg/kg, i.p., $n = 5$) compared with that by glibenclamide (△, 14.8 mg/kg, i.p., $n = 5$) (lower) in streptozotocin-diabetic mice. The values represent the means (%) + s.e.m. $^*P < 0.05$: Significant difference from saline-control (○, $n = 4$).

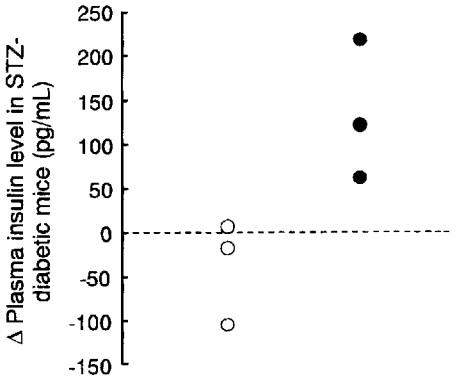

FIGURE 3 Increase in plasma insulin level (pg/mL) by gymnemic acid IV (13.4 mg/kg, i.p.) in streptozotocin-diabetic mice. The values represent the difference of values 2 h minus 0.5 h after injection of gymnemic acid IV (●) or saline (○) in each mice. Average values of plasma insulin level at 0.5 h in control group and gymnemic acid IV group were 455 pg/mL and 380 pg/mL, respectively.

TABLE I Glucose metabolism-related effects by gymnemic acids I, II, III, IV and gymnemasaponin V

	Antihyperglycemia	*Glucose uptake inhibition* [9]	*α-Glycosidase inhibition*	*Antisweet* [7]
Gymnemic acid I	—	—		○
Gymnemic acid II	—	○		○
Gymnemic acid III	—	—		○
Gymnemic acid IV	○	○	—	○
Gymnemasaponin V	—	—		

○: effective, —: no effect, and a vacant column: not tested.

The antihyperglycemic effect of gymnemic acid IV partly may be explained by the insulin-releasing action in pancreatic B cells of STZ-diabetic mice, because gymnemic acid IV increased the plasma insulin level of this diabetic mouse model possessing the B cells with ability of insulin release [10]. The mechanism of insulin-releasing action by gymnemic acid IV in STZ-diabetic mice has remained to be solved. Present results give a relevant elucidation for using an Indian traditional medicine, a prescription with leaves of *G. sylvestre* R. BR. to treat type 2 diabetic patients. In addition, gymnemic acid IV inhibits glucose uptake [9] but not α-glycosidase activity, and has antisweet activity [8] (Table I). The administration of gymnemic acids III and IV (100 mg/kg, p.o.), not other three compounds, tends to rather delay the recovery to pre-loaded levels of blood glucose in the glucose tolerance curve [11]. This may be an unfavorable side effect.

The crude extract suppresses sucrose-induced sweetness in human [12], and the antisweet activities of several gymnemic acids tend to increase with increase in the number of acyl groups because the activities of gymnemic acid I, II, XI and XII are more potent than those of gymnemic acid III, IV, VIII, IX and X [8,13]. On the other hand, a tigloyl group at R^1 position and a hydroxyl group at R^2 position seem to be important for antihyperglycemic action in gymnemic acid derivatives.

In conclusion, present results indicate that gymnemic acid IV may partly contribute to the antihyperglycemic effect by the leaves of *G. sylvestre*. Gymnemic acid IV may be an anti-obese and antihyperglycemic pro-drug.

EXPERIMENTAL SECTION

Animals

Male ddY mice (4 weeks old) and male Wistar rats (150–350 g weight, 5–6 weeks old) were used. Mice were singly injected with 150 mg/kg of STZ (Sigma, St. Louis, MO, USA) into the tail vein and were used 4 weeks after injection. These mice were used after fasting for 12–14 h before application of compounds. The fasted blood glucose level of the mice was 286.3 ± 14.8 mg/dL (mean $\pm$ s.e.m., $n = 23$).

Compounds Used

The methanol extract of the leaves of *G. sylvestre* (Lot No. 25889, Itou Kampo Co., Osaka) which were collected at Madurai in India was repeatedly separated by reverse-phase and normal-phase SiO_2 column chromatography to give a crude saponin fraction. It was successively purified by reverse-phase SiO_2 column chromatography and HPLC to furnish gymnemic acids I (0.012% from leaves), II (0.0086%), III (0.0091%), IV (0.0060%), and gymnemasaponin V (0.016%) [9]. Glibenclamide (Research Biochemicals Inc., MA) and acarbose (Bayer Pharmaceutical Co., Wuppertal, F.R.G.) were used as positive control. These compounds were suspended in saline or solubilized in DMSO, which was diluted finally to 5% with the nutrient solution.

Determination of Blood Glucose Levels in Mice

Under light anesthesia with ether, the blood sample (20 μL) of mouse was obtained from the orbital venous plexus using capillary glass tubes prior to

and 2, 4, 6 h after i.p. injection of each compound. The blood glucose levels were measured by the glucose oxidase method using a glucose analyzer (Beckman Instruments Inc., CA, USA).

Evaluation of Antihyperglycemic Activity

The antihyperglycemic activity of test compounds in STZ-diabetic mice was estimated, as previously reported [14]. It was evaluated as the percentage of one value (before–after) against another (before–85), where before and after represent a blood glucose level before and after injection of test compounds, respectively, and 85 (mg/dL) represents the mean blood glucose level in fasting state of normal mice.

Determination of Plasma Insulin Levels in Mice

Under anesthesia with ether, the blood samples were obtained from the orbital venous plexus using capillary glass tubes 30 min and 2 h after injection. The blood insulin levels were determined by an insulin ELISA kit (Morinaga Seikagaku Corporation, Tokyo, Japan).

Measurement of α-Glycosidase Activity in Rat

Brush border membrane vesicles prepared from rat small intestines were used to assay activities of sucrase and maltase (α-glycosidase) [15]. The reaction procedure by Dahlqvist [16] were modified. The substrate (37 mM maltose or sucrose), a test compound and the enzyme in 0.1 M maleate buffer (pH 6.0) were incubated together at 37°C. After 30-min incubation, 0.8 mL water was added to the test tube, and the tube was immediately immersed in boiling water for 2 min, then cooled with water. The concentration of D-glucose was determined by a glucose oxidase method.

Statistical Analysis

Significant differences between mean values before and after administration of a test compound or between mean values of saline control and treatment were statistically analyzed by Student's *t*-test. All data are the mean ± s.e.m.

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