

Antidiabetic Effect of Nitobegiku, the Herb *Tithonia diversifolia*, in KK-Ay Diabetic Mice

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Received March 9, 2005; accepted July 9, 2005

Nitobegiku (the herb of *Tithonia diversifolia* (HEMSL) A. GRAY) has been used as a medicinal plant for diabetes. The antidiabetic effect of an 80% ethanol extract of Nitobegiku (Td) was investigated in KK-Ay-mice, an animal model of type 2 diabetes. Td (500 mg/kg body weight) reduced the blood glucose of KK-Ay mice 7 h after a single oral dose. No change in blood glucose in Td-treated normal mice (ddY) was seen. Td (500 mg/kg) reduced blood glucose in KK-Ay mice 3 weeks after a single oral dose and also significantly lowered plasma insulin in KK-Ay mice under similar conditions. Td-treated KK-Ay mouse blood glucose was significantly decreased in an insulin tolerance test. These results support the hypothesis that Td improves glucose metabolism by reducing insulin resistance. Therefore, Nitobegiku may be useful for the treatment of type 2 diabetes.

Key words *Tithonia diversifolia*; antidiabetic effect; Nitobegiku; KK-Ay mice; insulin resistance

The plant kingdom is an important potential source of effective oral hypoglycemics. More than 400 species have been reported to display hypoglycemic effects, but only a few have been investigated in any detail.^{1–3)}

Nitobegiku, the herb *Tithonia diversifolia*, (*Chrysanthemum*) was used as a decoration for the emperor in China in ancient times.⁴⁾ Moreover, it has been used as a traditional medicine for diabetes (polyuria and polydipsia) in Taiwan.⁵⁾ We have described the hypoglycemic activity of the water extract of Nitobegiku given as a single dose.⁶⁾ However, there is no experimental evidence detailing improved hyperglycemia for repeated administration. In the present study, we examined the antidiabetic effect of an 80% ethanol extract of Nitobegiku (Td) using KK-Ay diabetic mice, an animal model of type 2 diabetes.

MATERIALS AND METHODS

Materials The traditional Taiwanese preparation, an 80% ethanol extract of the herb of Nitobegiku (Td), was obtained from Hiro International Co., Ltd., Tokyo, Japan (Lot No. E040325). A voucher specimen (herbarium No. 728796) is deposited at the herbarium of the National Science Museum (Tsukuba, Ibaraki, Japan). Nitobegiku was extracted with 80% ethanol in a heating bath (65 °C, 2 h). The 80% extract were lyophilized and stored at room temperature until use. The yield was 9.75%. Td contains 0.0037% tagitinin C.⁷⁾ For oral administration, Td (100, 500, 1500 mg/kg) was suspended in distilled water (20 ml). For subcutaneous administration, insulin (0.5 U/kg) was dissolved in saline (10 ml).

Animals and Treatments Adult male ddY strain mice (SLC, Shizuoka, Japan) weighing 22–25 g were used. The mice were housed in an air-conditioned room at 22±2 °C with a 12 h light and 12 h dark cycle. The animals were kept in the experimental animal room for 7 d with free access to food and water. Blood samples were withdrawn from the cavernous sinus with a capillary for glucose determinations.

KK-Ay strain male mice (6 weeks old, weighing 39–43 g) (Clea, Tokyo, Japan) with a blood glucose level above

250 mg/dl and considered to be diabetic were used. The animals were treated and housed under the same conditions as the ddY mice for 7 d. The experiments were started between 10:00–11:00 a.m.

Effect of a Single Dose of Td on Blood Glucose in KK-Ay Mice Td (100, 500, 1500 mg/kg) dissolved in 20 ml of distilled water was administered orally to the mice. The control group received an equal volume (20 ml/kg) of distilled water. Blood samples were taken for glucose determination 4 and 7 h later. This experiment was performed under nonfasting conditions.

Effect of 3 Week Repeated Administration of Td on Blood Glucose in KK-Ay Mice Td (500 mg/kg) suspended in 20 ml distilled water was administered orally once a day for 3 weeks to the mice. The control group received an equal volume (20 ml/kg) of distilled water. Blood samples were taken for glucose determination every week. This experiment was performed under nonfasting conditions.

Insulin Tolerance Test An insulin tolerance test was performed at the end of the repeated administration. After overnight fasting, an insulin (0.5 U/kg) solution was injected subcutaneously into the mice and blood samples were obtained for glucose determinations 0, 30, 60, and 120 min later.

Determination of Blood Glucose and Insulin Blood glucose levels in both normal and diabetic animals were determined by the glucose oxidase method⁸⁾ and plasma insulin was measured by a GLAZYME Insulin-EIA TEST.⁹⁾

Statistical Analysis All data are expressed as the mean±S.E.M. Student's *t* test and ANOVA were used for the statistical analysis. Values were considered to be significantly different when the *p* value was less than 0.05.

RESULTS

Effect of Td on Blood Glucose in KK-Ay Mice (Single Administration) The mean blood glucose levels of KK-Ay mice at various time intervals after a single oral administration of Td (100–1500 mg/kg) are shown in Fig. 1. Td (500 mg/kg, *p.o.*) lowered blood glucose 7 h after the admin-

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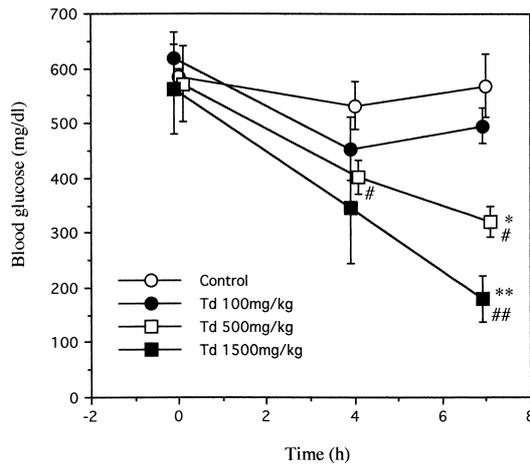


Fig. 1. Effect of a Single Dose of Td on Blood Glucose in KK-Ay Mice

Td (100, 500, 1500 mg/kg) was administered orally to the mice. The control mice received the same volume of distilled water. Blood samples were taken for glucose determinations 4 and 7 h later. Each value represents the mean \pm S.E.M. from 4–5 mice. Significantly different from control, * p <0.05, ** p <0.01 (by ANOVA). Significantly different from 0h, # p <0.05, ## p <0.01 (by ANOVA).

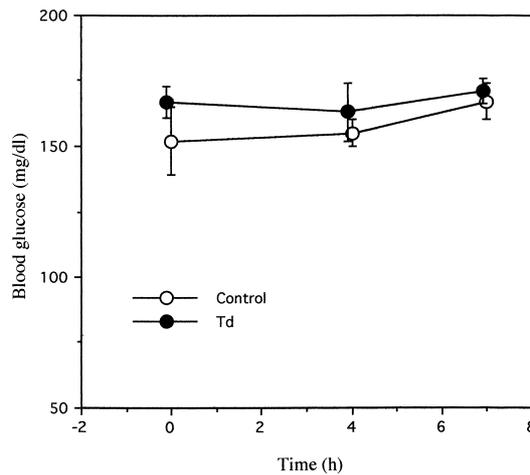


Fig. 2. Effects of a Single Dose of Td on Blood Glucose in ddY Mice

Td (1500 mg/kg) was administered to the mice at the same time and blood samples were taken for glucose determinations after 4 and 7 h. Each value represents the mean \pm S.E.M. from 5 mice.

istration (p <0.05). Td (1500 mg/kg)-treated mice showed a significant decrease in plasma glucose at 7 h compared with the control values (p <0.05) (Fig. 1).

Effect of Td on Blood Glucose in Normal Mice The effects of oral administration of Td on the blood glucose of normal mice are shown in Fig. 2. No differences in blood glucose were observed between the concentrations at 4 and 7 h after administration, when compared with the control values.

Effect of Repeated Oral Dosing of Td on Blood Glucose in KK-Ay Mice The mean blood glucose levels of KK-Ay mice at various week intervals after repeated oral dosing of Td (500 mg/kg) are shown in Fig. 3. Td (500 mg/kg, *p.o.*) lowered blood glucose 2 and 3 weeks after administration (p <0.05). The body weights of the Td-treated mice were not significantly different from those of the control mice (Fig. 4).

Effect of Td on Plasma Insulin in KK-Ay Mice The effect of Td in KK-Ay mice is shown in Fig. 5. The plasma in-

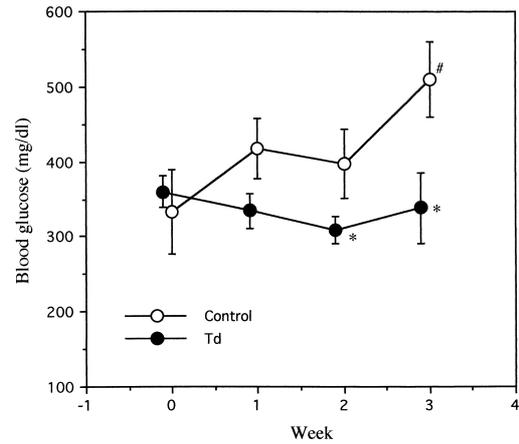


Fig. 3. Effects of Repeated Doses of Td on Blood Glucose in KK-Ay Mice

Td (500 mg/kg) was administered orally to the mice for 3 weeks. The control mice received the same volume of distilled water. Blood samples were taken for glucose determination every week. Each value represents the mean \pm S.E.M. from 5–7 mice. Significantly different from control, * p <0.05 (by ANOVA). Significantly different from 0 week, # p <0.05 (by ANOVA).

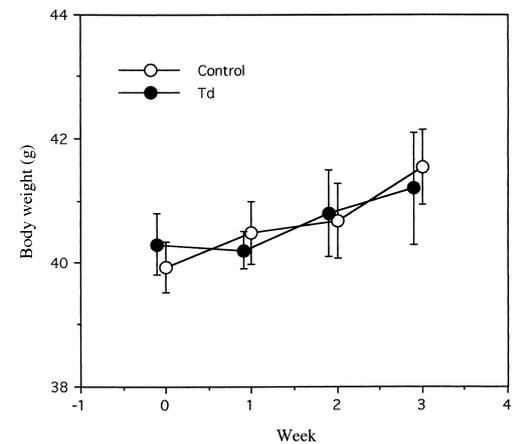


Fig. 4. Effects of Repeated Doses of Td on Body Weight in KK-Ay Mice

Td (500 mg/kg) was administered orally to the mice for 3 weeks. The control mice received the same volume of distilled water. Each value represents the mean \pm S.E.M. from 5–7 mice.

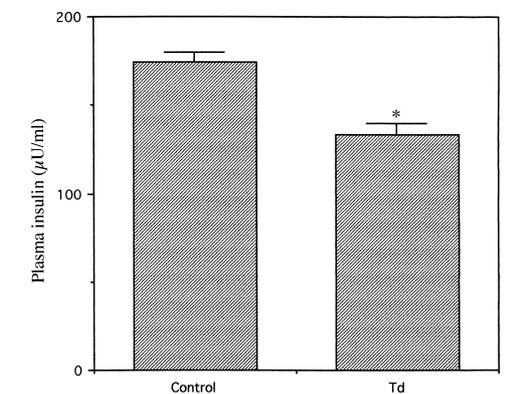


Fig. 5. Effects of Td on Plasma Insulin in KK-Ay Mice (3 Weeks)

Td (500 mg/kg) was orally administered to the mice and 3 weeks, blood samples were taken for insulin determination. Each value represents the mean \pm S.E.M. from 5 mice. Significantly different from control, * p <0.05.

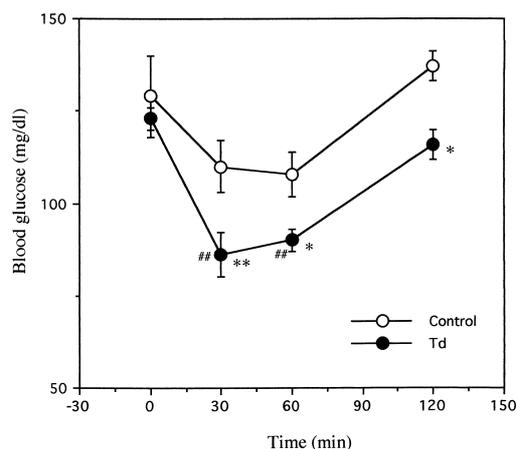


Fig. 6. Effects of Td on Blood Glucose in Insulin Tolerance Test

Td was administered to the KK-Ay mice 3 weeks after the administration, insulin (0.5 U/kg) was injected subcutaneously. Blood samples were taken for glucose determinations (0, 30, 60 and 120 min later). Each value represents the mean \pm S.E.M. from 5 mice. Significantly different from control, * $p < 0.05$, ** $p < 0.01$ (by ANOVA). Significantly different from 0 min, ## $p < 0.01$ (by ANOVA).

sulin level in Td-treated KK-Ay mice decreased 3 weeks after administration ($p < 0.05$) (Fig. 5).

Insulin Tolerance Test The insulin tolerance test for Td is shown in Fig. 6. Td-treated KK-Ay mice showed a significant decrease in blood glucose 30, 60 and 120 min after insulin administration compared with the control (30 min: $p < 0.01$, 60 and 120 min: $p < 0.05$).

DISCUSSION

The results of this study clearly show that an 80% ethanol extract of Nitobegiku (Td) produces a consistent hypoglycemic effect. We examined the dose-dependence (100, 500, 1500 mg/kg) after Td treatment, and observed antidiabetic activity at 500 and 1500 mg/kg after oral administration. Therefore, we examined the effect of repeated 100 mg/kg Td administration. We examined the therapeutic effects of Td on hyperglycemia in KK-Ay mice, an animal genetic model of type 2 diabetes mellitus. The KK mouse strain develops moderate degrees of obesity¹⁰ and diabetes that are especially apparent in animals older than 5 months of age and/or when fed a high-caloric diet.¹¹ Insertion of the lethal yellow agouti gene (Ay) into KK mice resulted in a congenic lethal yellow obese KK mouse strain, KK-Ay mice,¹² which are characterized by severe obesity, hyperinsulinemia, and insulin resistance, which are features of type 2 diabetes.¹³ Both KK and KK-Ay mice have provided useful

model systems with which to study the pathogenesis, therapy, and prevention of obesity and diabetes.¹⁴

Td decreased the blood glucose of KK-Ay mice and had no effect on blood glucose levels in normal mice, indicating that Nitobegiku is useful for type 2 diabetes. It seems likely that Td exerts its hypoglycemic activity after the metabolic process because Td lowered blood glucose 7 h after administration. Blood glucose levels following administration of the 80% ethanol extract of Nitobegiku were lower than those of the water extract. Repeated administration of Td resulted in hypoglycemia with reduced plasma insulin. These results indicate that Td improves hyperinsulinemia in type 2 diabetes. Insulin resistance in peripheral tissues is known to be one of the major pathogenic factors of type 2 diabetes. The finding that Td decreases blood insulin levels in KK-Ay mice is important.

Td-treated KK-Ay mice also had lower blood glucose levels in the insulin tolerance test and the hyperinsulinemia improved. Insulin (0.5 U/kg)-treated KK-Ay mice did not have lower blood glucose levels because of insulin resistance in the peripheral tissues, suggesting that Td lessens insulin resistance. Further studies will be needed to elucidate the mechanism of these effects. These results suggest the clinical use of Nitobegiku in the treatment of diabetes mellitus, especially type 2 diabetes, may be appropriate.

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