Byakko-ka-ninjin-to (BN) has been used as a traditional Oriental medicine for diabetes. Suzuki et al. reported on the antidiabetic activity of orally administered BN in KK-Ay mice. We also found that BN decreased blood glucose levels in KK-Ay mice. Moreover, the 50 and 100% methanol layers of BN decreased blood glucose, while the water layer did not change. One of the constituents of BN is the rhizoma of *Anemarrhena asphodeloides*. The rhizoma of *Anemarrhena asphodeloides* has been used as a traditional Oriental medicine for diabetes (polyuria and polydipsia) and constipation, and antidiabetic activity has been observed. However, because previous studies involved intraperitoneal administration, little is known about its oral antidiabetic activity. The purpose of this study therefore is to examine the antidiabetic activity after oral administration and the active components from the water extract of the rhizoma of *Anemarrhena asphodeloides* (AA) using a type 2 diabetic animal model and bioassay-guided fractionation.

**RESULTS**

**Effect of AA in Normal Mice**

No change in mean blood glucose levels in normal mice was observed after oral administration of AA (90 mg/kg), as shown in Fig. 1. However, mice that received tolbutamide (50 mg/kg body weight), a known sulfonylurea hypoglycemic agent, had reduced blood glucose levels 7 h after administration. AA-treated KK-Ay mice had significantly reduced blood glucose levels in an insulin tolerance test. Based on these results, the antidiabetic mechanism of AA may be due to decreased insulin resistance. In addition, the active components of AA were confirmed to be mangiferin and its glucoside.

**Key words** *Anemarrhena asphodeloides*; mangiferin; antidiabetic activity; KK-Ay mouse

**Effect of AA in KK-Ay Diabetic Mice**

The mean blood glucose levels in KK-Ay mice after oral administration of various doses of AA and tolbutamide are shown in Fig. 2. The hypoglycemic effect of AA was dose-dependent in KK-Ay mice. AA 90 mg/kg lowered blood glucose from the basal level of 570 ± 29 to 401 ± 59 mg/dl 7 h after administration ($p < 0.05$). Mice that received tolbutamide 50 mg/kg-treated had lower blood glucose levels ($p < 0.05$). The effects of AA on serum insulin level 7 h after administration are shown in Fig. 3. AA tended to decrease the serum insulin level in KK-Ay mice.

**Insulin Tolerance Test**

AA decreased blood glucose levels in KK-Ay mice 30 min after the administration of insulin when compared with baseline levels ($p < 0.01$). A significant decrease in blood glucose was observed in AA-treated mice 30 and 60 min after insulin administration as compared with the corresponding controls ($p < 0.05$) (Fig. 4).

**Effect of Water, 50% Methanol Fraction, and Methanol...**

*To whom correspondence should be addressed. e-mail: miura@suzuka-u.ac.jp © 2001 Pharmaceutical Society of Japan*
The effect of water, and the 50% methanol and methanol fraction of the rhizoma of *Anemarrhena asphodeloides* water extract (AA) are shown in Fig. 5. The 50% methanol fraction reduced blood glucose levels in KK-Ay mice. The MeOH fraction tended to decrease blood glucose.

**Effects of Mangiferin and Mangiferin-7-O-beta-glucoside in KK-Ay Mice** The active 50% methanol fraction of AA was analyzed by high-performance liquid chromatography (HPLC). The structures of mangiferin (MF) and mangiferin-7-O-beta-glucoside (MG) were confirmed by spectroscopic methods.\(^5\) MF and MG (Fig. 6) showed similar antidiabetic activity at a dose of 90 mg/kg (\(p<0.001\)) (Fig. 7). Seven hours after the administration of MF 90 mg/kg, the blood glucose level was in the range of 56% of the baseline value.

**DISCUSSION**

The results of the present study confirmed that orally administered AA has consistent antidiabetic activity in KK-Ay mice, an animal model of type 2 diabetes mellitus. KK-Ay mice are known to develop genetically, diabetes like ob/ob mice and New Zealand obese mice.\(^6\) Hyperinsulinemia occurs as a result of insulin resistance. In a preliminary study, we examined time dependence (0, 4, 7 h) after administration of AA to KK-Ay mice, and found that the lowest blood glucose levels were achieved 7 h after oral administration (un-
published observation). AA 90 mg/kg had no effect on blood glucose levels in normal mice without insulin resistance. AA tended to decrease serum insulin levels in KK-Ay mice. Moreover, AA decreased blood glucose levels in the insulin tolerance test. These results indicate that the antidiabetic mechanism of AA may be due to decreased insulin resistance. AA appears to have little toxicity (LD50 = 900 mg/kg) (unpublished observation). Moreover, mice that received AA 900 mg/kg did not show any obvious stimulatory effects, suggesting that AA was not toxic. In addition, we have identified the active fractions associated with the antidiabetic effect as MF and its glucoside MG. Little is known about the antidiabetic activity of xanthone compounds and why they have a significant antidiabetic effect.

Further studies are needed to clarify the details. The above experimental results suggest that the antidiabetic effect of MF and its glucoside MG support the traditional medical use of Anemarrhena asphodeloides.

MATERIALS AND METHODS

Materials The rhizoma of Anemarrhena asphodeloides was obtained from Tsumura Co., Tokyo, Japan. AA (crude powder extract) 5.5 g contains spray-dried aqueous extracts (100 °C, 2 h, washed twice with 2.01 of water). The crude powder extract was stored at room temperature until use. The extract yield was 23.5%. The typical constituents of Anemarrhena asphodeloides are xanthones (MF 1.53%, MG 0.45%). These compounds were isolated by conventional method as previously reported.5)

The active component of AA was isolated by bioassay-guided fractionation, as summarized in Chart 1. The water extract of AA was chromatographed on a diaion HP-20 column using water, 50% methanol, and methanol (elution volume 2.0 l). Each fraction was evaporated. The 50% MeOH fraction of AA (active layer) was analyzed by HPLC (MeCN : H2O : 85% phosphoric buffer = 10:89:1, ODS column length 250 mm, diameter 46 mm, flow rate 0.8 ml/min) (tR MF = 36.8, MG = 16.1 min) (Fig. 6). The structures of MF and MG were confirmed by spectroscopic methods.5)

Animals Normal male (6-week-old) and KK-Ay (12-week-old) mice were used in this study. 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. For the determination of blood glucose levels, blood samples were drawn from the cavernous sinus with a capillary. Tolbutamide, used as a positive control, was obtained from Sigma Co., Ltd., Tokyo, Japan.

Insulin Tolerance Test After overnight fasting (18 h), insulin (0.5 U/kg body weight) solution was administered subcutaneously. Blood samples were collected before insulin administration and 30, 60, and 120 min thereafter.

Determination of Blood Glucose Blood glucose levels in diabetic animals were determined by the glucose oxidase method.7) All data were expressed as mean ± S.E. Student's t-test was used for statistical analysis. The values were considered to be significantly different when the p value was 0.05.

REFERENCES


Fig. 7. Effects of MF and MG in KK-Ay Mice
Each compound (90 mg/kg) was administered orally to KK-Ay mice. After 7 h, blood samples were taken for blood glucose level determination. Data shown are expressed as the mean ± S.E. for 4—5 mice/group. Significantly different from baseline, * p<0.05, ** p<0.001.