Hypoglycemic Effect of Water Extract of the Root of *Pandanus odoratus* RIDL.

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Hypoglycemic effect of water extract of the root of *Pandanus odoratus* RIDL. (Thai name: Toeï-hom, Pandanaceae) was examined in normal and streptozotocin-diabetic rats. In the hypoglycemic test without glucose load, an administration of the extract at doses of 0.125–0.5 g/kg *p.o.* did not affect significantly the plasma glucose level in normal rats, whereas the extract significantly lowered the plasma glucose level at a dose of 0.5 g/kg *p.o.* in diabetic rats. In oral glucose tolerance test, an administration of the extract at a dose of 0.5 g/kg *p.o.* significantly lowered the plasma glucose level in normal rats. The extract at doses of 0.5 and 1.0 g/kg *p.o.* also significantly lowered the plasma glucose level in diabetic rats. A reference drug, glibenclamide at a dose of 5 mg/kg *p.o.* showed a significant hypoglycemic effect in both normal and diabetic rats.

Repeated administration of the extract at doses of 0.25 and 0.5 g/kg *p.o.* for 7 d produced a significant hypoglycemic effect in diabetic rats. Glibenclamide (5 mg/kg *p.o.*) also caused a significant hypoglycemia in the diabetic rats.

LD₅₀ (95% confidence limit) after intraperitoneal injection was 1.87 (1.26–2.76) g/kg in male and female rats and 1.62 (1.18–2.24) g/kg in male and female mice, respectively. The LD₅₀ after oral administration was over 8 g/kg in both sexes of rat and mice.

Key words *Pandanus odoratus*; hypoglycemic effect; oral glucose tolerance test; streptozotocin; diabetes

Pandanus odoratus RIDL. (Fragrant screw pine, Thai name: Toeï-hom, Pandanaceae) is an erect shrub, 0.5–1 m high, the stem bearing a few prop roots. The various parts of Toeï-hom are used in food and traditional medicine. The juice from bruised fresh leaves is used as a green colourant in Thai desserts. The fresh leaves also contain aromatic oils which are claimed to be cardiogenic.¹⁾ The root and rhizome are believed by patients and traditional practitioners to be effective against diabetes.²⁾

The water decoction of Toeï-hom root and rhizome has been traditionally used in diabetic patients without any scientific evidence. Peungvicha *et al.* reported that oral administration of water extract of the root and rhizome at 1, 2 and 4 g/kg significantly decreased the plasma glucose level in normal female rats.³⁾ The higher the doses given, the greater were the decreases in plasma glucose levels and the longer were they observed. The extract at doses of 2 and 4 g/kg also significantly decreased the plasma glucose level in mild and severe alloxan-diabetic female rats.⁴⁾

To save resources because rhizome is an important part in the harvesting of this plant, we used Toeï-hom root without rhizome for the extraction and investigated whether it had hypoglycemic activity in normal or streptozotocin-diabetic rats. The acute toxicity (LD₅₀) of the extract was also investigated.

MATERIALS AND METHODS

Plant Material The authentic samples of root of *Pandanus odoratus* RIDL. used in this study were identified by the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand.

Extraction Dry powder of the root of *Pandanus odoratus* RIDL. (300 g) was macerated with 3 l of water at 70 °C for 1 d and then filtered. The extract was lyophilized

after evaporating at 70 °C until the volume was reduced to about 400 ml. The lyophilized extract was a brown bulky powder weighing about 26 g (yield = 8.7%) and this was kept in a cool place until used.

Animal Male Wistar rats, five weeks old (weighing 120–140 g), obtained from SLC (Shizuoka, Japan) were housed with free access to food and water for at least one week in an air-conditioned room (23 ± 1 °C with 55 ± 5% humidity), and subjected to a 12 h light/dark cycle prior to experiments.

Diabetic Induction Streptozotocin (75 mg/kg) was intraperitoneally injected to rats after overnight fasting to induce experimental diabetes. The diabetic condition was checked daily using a urine glucose strip throughout the experiment.

Experimental Procedure 1) Hypoglycemic effect was examined by the test without glucose load and by the oral glucose tolerance (OGT) test in normal and streptozotocin-diabetic rats. Rats were divided into 5 groups: control, glibenclamide and Pandanus extract (three doses). Prior to the experiment, rats were fasted for 15 h. Then, distilled water (control), glibenclamide or Pandanus extract was orally administered to each group of animals.

For the hypoglycemic test without glucose load, blood samples were taken at 0 (just before administration), 30, 60, 90, 120, 150 and 180 min after the administration of water, glibenclamide or Pandanus extract for glucose assay.

For the OGT test, thirty minutes later, glucose (2.5 g/kg) was orally given to each rat 30 min after the administration of water, glibenclamide or Pandanus extract. Blood samples were taken from the tail vein at –30 (just before the administration of the extract), 0 (just before glucose administration), 30, 60, 90, 120, 150 and 180 min for glucose assay.

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2) Repeated administration of the extract in diabetic rats: Rats were induced to be experimentally diabetic by 75 mg/kg streptozotocin after overnight fasting. Two days later, non-fasting plasma glucose level of each rat was measured and 7–9 hyperglycemic rats were selected and divided into 4 groups (day 0). Then, water (control), glibenclamide or the extract was given once daily for 7 d and blood samples from tail vein were taken at day 8 (15 h after the last administration) for the plasma glucose assay.

3) Blood glucose analysis: Plasma glucose concentration was determined by means of the PGO enzyme method⁵⁾ and by measuring the optical density at the 505 nm wavelength with a spectrophotometer (Beckman DU 68).

4) Acute toxicity: Number of deaths within 7 d after oral or intraperitoneal administration of the *Pandanus* extract was examined in both sexes of rats and mice, and LD₅₀ was calculated.

Chemical Streptozotocin (Sigma) was dissolved in citrate buffer (pH 7.45) and glibenclamide (Euglucon®) was dissolved in distilled water. Glucose oxidase kit (Wako Chemicals Ltd.) was used for the measurement of plasma glucose level.

Statistical Analysis The statistical analysis was performed by ANOVA followed by Dunnett test. The plasma glucose levels were expressed as the mean $\pm$ standard error.

RESULTS

Hypoglycemic Test in Normal and Streptozotocin-diabetic Rats Effect of water extract of the root of *Pandanus odoratus* on fasting plasma glucose levels in normal and streptozotocin-diabetic rats without glucose load is shown in Fig. 1. In normal rats the extract did not produce a significant decrease in plasma glucose level except at 150 min after oral administration of 0.5 g/kg. In diabetic rats, however, the extract produced a significant decrease as compared with the control at 90 and 150 min after the administration of 0.25 g/kg and at 90, 120 and 150 min after the administration of 0.5 g/kg. The reference drug, glibenclamide (5 mg/kg, *p.o.*), caused a significant decrease in plasma glucose level at 90 to 180 min and at 60 to 180 min in normal and diabetic rats, respectively.

OGT Test in Normal and Diabetic Rats Effect of water extract of the root of *Pandanus odoratus* on plasma glucose levels in normal and streptozotocin-diabetic rats loaded with oral glucose is shown in Fig. 2. In both groups, plasma glucose level reached a peak at 30 min and gradually decreased to pre-glucose load level. In the normal rat, the extract produced a significantly lower plasma glucose level than those of the control at 90 and 120 min and at 120 min after glucose load, at a dose of 0.5 g/kg and a dose of 1.0 g/kg, respectively. Glibenclamide (5 mg/kg) produced a significant decrease in the plasma glucose level at 90 through 180 min.

In diabetic rats, the plasma glucose level was three times higher than those of normal rats. Oral administration of 0.5 g/kg of the extract produced a significant decrease in the plasma glucose level at 90, 120 and 150 min, as compared with the control, while 0.25 and 1 g/kg of

Fig. 1. Effect of Oral Administration of Water Extract of Root of *Pandanus odoratus* on the Fasting Plasma Glucose Levels in Normal and Diabetic Rats

—■—, control ($n=8, 6$); —●—, glibenclamide ($n=7, 7$); —△—, P 0.125 g/kg ($n=5, 6$); —○—, P 0.25 g/kg ($n=6, 7$); —□—, P 0.5 g/kg ($n=6, 7$). Water (control), *Pandanus* extract or glibenclamide was administered orally at 0 min after 15 h fasting. * $p<0.05$, ** $p<0.01$ compared with the control. Number of experiments in diabetic and normal rats is shown in parenthesis. Vertical bar shows the mean $\pm$ S.E.M.

Fig. 2. Effect of Oral Administration of Water Extract of Root of *Pandanus odoratus* on OGT Test in Normal and Diabetic Rats

—■—, control ($n=8, 10$); —●—, glibenclamide ($n=7, 8$); —△—, P 0.25 g/kg ($n=6, 7$); —○—, P 0.5 g/kg ($n=8, 7$); —□—, P 1.0 g/kg ($n=7, 7$). Water (control), *Pandanus* extract or glibenclamide was orally administered at -30 min and 1.25 g/kg glucose was loaded at 0 min after 15 h fasting. * $p<0.05$, ** $p<0.01$ compared with the control. Number of experiments in diabetic and normal rats is shown in parenthesis. Vertical bar shows the mean $\pm$ S.E.M.

the extract produced a significant decrease in the level at 90 and 120 min, respectively. Glibenclamide produced a significant decrease in the level only at 90 min.

Repeated Administration of the Extract in Diabetic Rats The extract at doses of 0 (water), 0.25 and 0.5 g/kg *p.o.* was administered to diabetic rats once daily for 7 d and non-fasting plasma glucose level was determined at day 8. The repeated administration of the extract produced a significant decrease in plasma glucose level as compared with the day 8 control level, although the water group showed a significant increase in the level as compared with the pretreatment level. Glibenclamide (5 mg/kg, *p.o.*) also

Fig. 3. Effect of Repeated Administration of Water Extract of Root of *Pandanus odoratus* (P) in Diabetic Rats

* $p < 0.05$ compared with the control, day 0. ** $p < 0.01$ compared with the control, day 8. Number of experiments is shown in the column.

significantly decreased the plasma glucose level in comparison with the day 8 control (Fig. 3).

LD₅₀ of the Extract LD₅₀ after oral administration of the extract was more than 8 g/kg (maximal dose examined) in rats and mice. LD₅₀ (95% confidence limit) after intraperitoneal injection was 1.87 (1.26–2.76) g/kg in male and female rats, and 1.62 (1.18–2.24) g/kg in male and female mice, respectively.

DISCUSSION

The hypoglycemic effect of the extract of *Pandanus* root appears to be more potent than that of the root and rhizome. The root extract produced hypoglycemia at a dose of 0.5 g/kg in the present study, whereas the root and rhizome extract showed the effect at doses over 1 g/kg.^{3,4)} It is possible that active principles are contained more in the root than in the rhizome.

It was apparent that in normal rats the plasma glucose levels decreased much more in the glibenclamide group than in the other groups. Glibenclamide, a sulphonylurea derivative commonly used as a hypoglycemic drug, significantly lowered the plasma glucose from 90 min through 180 min of the experiment. This acute action was caused by a stimulation of insulin secretion and inhibition of glucagon secretion.⁶⁾

It seemed that the root extract caused a cumulative hypoglycemic effect after repeated administrations. Single administration of a low dose (0.25 g/kg) of the extract did not produce this effect, although repeated administration

produced a significant effect. Theoretically, the blood or plasma glucose level after glucose load depends on factors like intestinal motility,⁷⁾ glucose absorption,⁸⁾ insulin secretion and metabolic factors⁹⁾ or glucose utilization. In *Pandanus odoratus*, a mechanism of action may not be due to the inhibition of glucose absorption. The reason is that the peak of the glucose tolerance curve (Fig. 1) which reflects the glucose absorption was the same in the control group and the extract group. Furthermore, the extract did not inhibit glucose absorption in the mouse jejunum *in vitro*.¹⁰⁾ The mechanism of action is still not known, however, and further investigations are necessary to reach a definite conclusion.

The presence of flavonoid compounds and coumarin in the root and rhizome extract was reported.¹¹⁾ The known pharmacological effect of coumarin is anti-coagulation.⁶⁾ Phytochemical screening test showed only the presence of coumarin, and it did not show the amount and whether or not it was adequate to show its pharmacological action.

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