Screening of traditional medicines for their hypoglycemic activity in streptozotocin (STZ)-induced diabetic rats and a detailed study on *Psidium guajava*

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Abstract

Some natural drugs were screened for their hypoglycemic activity in STZ-induced diabetic rats. Nine of them *viz. Ficus bengalensis, Filicium decipiens, Leucas cephalotes, Matteuccia orientalis, Morus insignis, Psidium guajava, Swertia japonica, S. chirayita,* and *Tinospora cordifolia,* showed a significant hypoglycemic activity when compared with control. Water extract of *P. guajava* showed a strong hypoglycemic activity in STZ-induced diabetic rats. So it was fractionated according to molecular size by dialysis and ultrafiltration, and hypoglycemic activity examinations of each fraction suggested the active component to be a glycoprotein with the molecular size of 50,000 to 100,000. The activity of leaves of *P. guajava* was found to be more potent than that of sprout. The water extract of *P. guajava* also significantly lowered the blood triglyceride level. The fraction with molecular size larger than 50,000 showed a significant dose dependent 2-DOG uptake stimulating activity in Rat 1 fibroblasts.

Key words Hypoglycemic activity, *Psidium guajava,* Myrtaceae, diabetic rats, 2-deoxy-D-glucose uptake, Rat 1 fibroblasts, streptozotocin.

Abbreviations 2-DOG, 2-deoxy-D-glucose ; STZ, streptozotocin ; BSA, bovine serum albumin ; FCS, fetal calf serum ; HIRc-B, human insulin receptor cell ; PGW100, glycoprotein with molecular size larger than 100,000 from *Psidium guajava* water extract ; PGW>50, glycoprotein with molecular size larger than 50,000 from *Psidium guajava* water extract ; PGW<50, glycoprotein with molecular size smaller than 50,000 from *Psidium guajava* water extract.

Introduction

The lack or insufficient insulin causes metabolic disorders resulting in high level in blood glucose all the time, commonly called diabetes mellitus. It has been estimated that 100 million of the people on the earth are suffering with diabetes mellitus and it is the third leading cause of death.[1] Unfortunately, there is no perfect treatment for diabetes in spite of development of scientific knowledge so far we have. On the other hand, there are some traditional drugs which are reported for the treatment of diabetes[2] but no sufficient pharmacological study about them is available till now. Regarding all these facts, an attempt was made to find the natural drugs having hypoglycemic activity. For this, STZ-induced diabetic rats were prepared, then blood sugar levels were examined before and after drug administration.

Twenty-three natural medicines were screened

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for their hypoglycemic activity, nine of them showed a significant activity. We had already reported the hypoglycemic activity of *M. insignis, M. orientalis, S. japonica* and *S. chirayita* and the active principles from *M. orientalis* and *S. japonica*.[3-6] In the present paper we wish to report the hypoglycemic activity of *P. guajava* and its effect on blood insulin and triglyceride levels together with 2-DOG uptake stimulating activity on HIRc B cells.

Materials and Methods

Cells and reagents : HIRc-B cells used in 2-DOG uptake experiment were kindly supplied by Dr. J. M. Olefsky. Bovine serum albumin (BSA), streptozotocin (STZ), trypsin and gentamycin were from Sigma Chemical Co., St. Louis, MO, USA ; heparin and glutamine were from Wako Pure Chemical Industries, Ltd., Osaka Japan ; tolbutamide was from Chugai Pharmaceutical Co. Ltd., Tokyo, Japan and buformin was from Kodama Co. Ltd., Tokyo, Japan. Dulbecco's Modified Eagle Medium (DMEM) and Dulbecco's phosphate buffer saline {PBS-(-)} were from Nissui Pharmaceutical Co. Ltd., Tokyo, Japan. Fetal calf serum (FCS) was from Gemini Bioproduct Inc., Calabasas, CA, USA ; ^{3}H-2-DOG (1132.2 GBq/mmol) was from (New England Nuclear) Du-Pont Co. Wilmington, DE, USA, and ^{125}I-insulin and Insulin Kit (Biotrek RPA 547) were from Amersham Japan Co., Ltd., Tokyo, Japan. Cell cultured materials were from (Falcon) Beckton Dickinson Co., NJ, USA. The glucose, cholesterol and triglyceride levels in the blood samples were analyzed using Reflotron kits from Boehringer Mannheim Co., Ltd., Tokyo Japan. All other chemicals were of analytical grade.

Plant materials : The leaves of *Morus insignis* BUR. (Moraceae) were collected in Argentina and the rhizomes of *Matteuccia orientalis* (HOOK.) TREV. (Aspidiaceae) were collected in Yatsuo, Toyama Prefecture, Japan. *Swertia japonica* MAKINO (Gentianaceae) was collected in Tohoku District of Japan, supplied by Uchida Pharmaceutical Co. Ltd., Japan and the sample of *S. chirayita* KARSTEN (Gentianaceae) was commercially available in New-Delhi, India. *Ficus bengalensis* L. (Moraceae), *Leucas cephalotes* SPRENG. (Labiatae), *Asparagus racemosus* WILLD. (Liliaceae) and *Aegle marmelos* CORR. (Rutaceae) were collected in the central part of Nepal. *Tephrosia candida* DC. (Leguminosae), *Alternanthera philoxeroides* GRISEB. (Amaranthaceae), *Filicium decipiens* THW. (Burceraceae) and *Rhizophora mucronata* LAM. (Rhizophoreae) were collected in Indonesia and *Tecomella undulata* (G. DON) SEEM. (Bignoniaceae) and *Caralluma tuberculata* (Asclepiadaceae) were collected in Pakistan. *Tinospora cordifolia* (WILLDENOW) MIERS (Minispermaceae) was collected in Myanmar. The sprout of *Psidium guajava* L. (Myrtaceae) was collected in Okinawa, Japan and the leaves of *P. guajava* L. in China. The other plant drugs, *Orthosiphon stamineus* BENTH. (= *Ocimum grandiflorum* BLUME) (Labiatae), *Gymnema sylvestre* (RETZ.) SCHULT. (Asclepiadaceae), *Ocimum basilicum* L. (Labiatae), *Equisetum arvense* L. (Equisetaceae), *Glechoma hederacea* L. (Labiatae), *Zea mays* L. (Graminae) were supplied by Yamanouhi Pharmaceutical Co. Ltd., Japan. These drug samples were identified morphologically and the voucher samples were preserved in Museum of Materia Medica of Toyama Medical and Pharmaceutical University, Toyama, Japan.

Preparation of extracts for hypoglycemic activity : In general, crude drugs (100 g) were extracted with 70 % ethanol (900 ml × 3) by refluxing for 3 h in first extraction and 2 h in each second and third extractions. The hot extract was filtered through filter paper and the combined filtrate was concentrated *in vacuo*, and lyophilized to obtain alcoholic extract which was used in animal experiments. Water extract was obtained by extracting the fresh drug with distilled water by the method described above. The extraction of *M. orientalis, S. japonica, S. chirayita* and *M. insignis* had already been discussed in the previous papers.[3-6] The samples (100 g) of *P. guajava, O. stamineus, G. sylvestre, O. basilicum, E. arvense, G. hederacea* and *Z. mays* were extracted with water by the general method, which yielded 22.7, 23.0, 15.4, 35.9, 18.5, 30.1 and 12.5 % as water extracts, respectively. *F. bengalensis, L. cephalotes, A. racemosus* and *A. marmelos* yielded 9.4, 13.5, 50.2 and 26.5 % as ethanol extracts and 1.5, 5.9, 8.6 and 10.6 % as water extracts, respectively. *S. miltiorrhiza* gave 26.1 % as water extract and 15.9 % as methanol extract on successive extractions. The successive extraction of *T. cordifolia* with $CHCl_3$, MeOH and water gave as $CHCl_3$ (6.2 %), MeOH (3.6 %), and water (1.9 %) extracts. All twenty-three crude drug extracts were tested for their hypoglycemic activity in STZ-induced diabetic rats.

Fractionation of water extract from P. guajava by dialysis : Water extract (9 g) was treated with distilled water (300 ml) and kept at 60°C for 2 h and the insoluble portion was separated by centrifugation. The supernatant was applied into the cellophane tube, dialyzed against distilled water and the water outside was changed three times at an interval of 24 h. The dialyzable (6.22 g) and undialyzable (480 mg) portions were considered as the fraction with molecular size smaller than 50,000 (PGW<50) and larger than 50,000 (PGW>50), respectively.

Fractionation of P. guajava by ultrafiltration : One kilogram each of leaves or sprout was submerged with distilled water (10 L) for 30 min at 95°C. The filtrate (8 L) was centrifuged at 10,000 g for 10 min and the supernatant was subjected to ultrafiltration (UF-LMS II, UF-100PS) to obtain the fraction (PGW 100) with molecular size larger than 100,000. The yields from the leaves and the sprout were 4.4 and 4.7 %, respectively. PGW 100 and PGW>50 were found to be a glycoprotein since they showed positive reactions for phenol-H_2SO_4 and Lowry tests.

Animals and treatment : Male Sprague-Dawley rats, 5 weeks of age, weighing 120-140 g were purchased from the Shizuoka Laboratory Animal Center (Japan) and maintained under 12 h light/dark cycle in a temperature and humidity controlled room. The animals were fed with a laboratory pellet chow (CLEA Japan Inc., Tokyo ; protein 24.0 %, lipid 3.5 %, carbohydrate 60.5 %) and given water *ad libitum*. Diabetes was induced in 12-14 h fasted rats by a single intravenous injection of 50 mg/kg of STZ[7,8] in 10 mM citrate buffer (pH 4.5) at about 10 : 00 a.m. The blood glucose level was checked on the third and the fourth days after injecting STZ. Animals having high blood glucose level (more than 300 mg/dl and less than 550 mg/dl) were considered as diabetic ones and divided into groups. The group treated with a mixture of tolbutamide (200 mg/kg) and buformin (1 mg/kg) was taken as a positive control group.[9] The group treated with an equal volume of physiological saline was taken as a negative control group and the rest ones were treated with extracts of different drugs. The blood glucose level lowering effects were evaluated after administering drugs *i.p.* or *p.o.*, five times. The drugs were administered twice a day at about 9 : 00 and 16 : 00 on the first and second days while on the third day, drugs were administered at 9 : 00 and blood sample were collected at about 15 : 00. In all experiments, the drugs were made as a suspension or a solution in physiological saline with or without 2 % acacia gum (w/v). Blood samples were syringed out through tail veins, immediately transferred into plastic tubes rinsed with heparin. The glucose level of blood samples were analyzed within an hour and triglyceride and cholesterol levels were analyzed within 3 h after sampling by the use of Reflotron kits.

Insulin assay : The STZ-induced diabetic rats were prepared by the procedure described above and were treated with PGW>50, five times with a dose of 50 mg/kg, twice a day, *i.p.* Blood samples were collected in a tube rinsed with heparin after decapitation. Plasma was separated by centrifugation (3000 rpm, 10 min) and the samples were frozen at 20°C until the assay. The immunoreactive insulin in plasma samples was measured by radioimmunoassay (RIA) method using Biotrak Rat Insulin Kit (RPA 547),

2-DOG uptake activity : The Rat 1 fibroblast cells expressing 1.2×10^6 insulin receptors (HIRc-B)[10] were cultured in DMEM with 10 % FCS. Confluent monolayer of HIRc-B (10^6 cells/well) in six multiwell dishes were refed with complete medium 16 h before the experiment. The medium was replaced by Krebs Ringer phosphate and HEPES buffer containing 0.5 % BSA and incubated for 20 min at 37°C. Cells were incubated with insulin (2, 5 and 20 nM) or PGW>50 (0.01, 0.1, 1.0 and 10 μg/ml) for 40 min at 37°C. Then cells were allowed to feed 2 mM ^3H-2-DOG (14.8 KBq/well). Five minutes later, the reaction was terminated, and washed three times with PBS. Cells were solubilized with 1 M NaOH and counted for ^3H after neutralization. Total protein of cells was analyzed by the methods of Lowry *et al.* taking albumin as a standard.[11]

Statistical analysis : All values expressed as mean ±S.E. were obtained from *n* number of experiments. The Student's *t*-test for unpaired observation between control and experimental samples was carried out for statistical evaluation of a difference ; p values of 0.05 or less were considered as statistically significant. The blood glucose level after drug administration was expressed as percentage with respect to the blood glucose level before drug administration by comparing with control.

Results and Discussion

We examined hypoglycemic activity of twenty-three crude drugs collected in different countries. Most of these drugs have been reported for the treatment of diabetes traditionally. The result of the

Table I Effects of extracts of crude drugs (each 5 times, twice a day) on blood glucose level in STZ-induced diabetic rats.

Group	Part used	Dose (mg/kg)	(n)	Blood glucose level (mg/dl) before drug[a]	after drug	Decrease (%)[b]
Control	......	......	50	422.9±28.6	454.8±20.3	...
Positive control	......	......	6	429.2±42.4	294.0±22.4	36.37*
Aegle marmelos	fruit					
MeOH extract		200 (*p.o.*)	5	392.8±32.3	396.4±20.2	6.13
Water extract		200 (*p.o.*)	5	393.8±31.4	377.0±40.4	10.90
Alternanthera philoxeroides	leaf[c]	100 (*i.p.*)	4	464.2±30.6	352.0±59.2	29.51
Asparagus racemosus	root					
Ethanol extract		100 (*i.p.*)	5	433.8±20.7	467.2±10.7	-0.10
Ethanol extract		200 (*p.o.*)	5	410.8±26.8	414.0±24.9	6.25
Water extract		200 (*p.o.*)	5	416.8±31.6	455.2±44.0	-1.58
Caralluma tuberculata	leaf[c]	100 (*i.p.*)	5	482.4±24.0	454.0±59.0	12.49
Ficus bengalensis	bark					
Ethanol extract		100 (*i.p.*)	4	428.8±20.9	137.8± 6.0	70.13**
Water extract		100 (*i.p.*)	4	436.6±20.0	134.8±13.8	71.34**
Filicium decipiens	leaf[c]	100 (*i.p.*)	5	460.4±19.5	208.6±44.5	57.88**
Glechoma hederacea	leaf[d]	100 (*i.p.*)	5	432.8±22.6	459.5±16.8	1.30
Gymnema sylvestre	leaf[d]	100 (*i.p.*)	5	391.2±42.1	394.8±16.1	6.13
Leucas cephalotes	whole plant					
Ethanol extract		100 (*i.p.*)	4	430.0±22.3	251.5±55.1	45.67**
Water extract		100 (*i.p.*)	4	433.8±42.0	233.0±76.4	50.04**
Ocimum basilicum	leaf[d]	100 (*i.p.*)	5	394.4±31.9	382.0±26.6	9.95
Orthosiphon stamineus	leaf[d]	100 (*i.p.*)	5	393.8±39.9	371.4±35.5	12.27
Psidium guajava	leaf					
Ethanol extract		100 (*i.p.*)	5	435.0±47.9	459.3±21.2	-1.78
Water extract		100 (*i.p.*)	5	434.2±24.4	134.3±14.5	71.25**
Rhizophora mucronata	leaf[c]	100 (*i.p.*)	2[e]	466.2±20.6	477.5±23.5	4.78
Salvia miltiorrhiza	root					
MeOH extract		100 (*i.p.*)	5	464.8±15.0	507.6±41.6	-1.58
Water extract		100 (*i.p.*)	5	467.2±10.7	474.0±16.2	5.67
Tecomella undolata	leaf[c]	100 (*i.p.*)	5	495.2±17.1	464.8±23.9	12.72
Tephrosia candida	leaf[c]	100 (*i.p.*)	4	466.8±50.0	312.3±83.8	37.79
Tinospora cordifolia	stem					
MeOH extract		100 (*i.p.*)	5	427.0±20.3	430.4±23.5	6.32
Water extract		100 (*i.p.*)	5	435.7±20.6	308.0±17.5	34.32**

Results are expressed as mean±S.E., Significantly different from control value, $^{**}p<0.01$, $^{*}p<0.05$. Positive control groups were administered 5 times of a mixture of 200 mg/kg of tolbutamide and 1 mg/kg of buformin, *i.p.* in the STZ-induced diabetic rats. a) Glucose level before administering the drugs or saline. b) Decrease in blood glucose level relative to the level before administration of drugs, expressed in % in comparison to control. c) Ethanol extract. d) Water extract. e) Three experimental animals were died.

hypoglycemic activity of these drugs in STZ- induced diabetic rats are shown in Table I. It has been reported that in some cases, a mixture of two hypoglycemic agents, tolbutamide and buformin is very effective for the treatment of diabetes.[9] Hence in the present study, a mixture of tolbutamide (200 mg/kg) and buformin (1 mg/kg) was taken as the positive control and it was found to be very effective than tolbutamide alone. Hypoglycemic activity was evaluated after treating five times with the drugs in each case since the results were more significant than the effect of single dose treatment in the preliminary examination. Nine of them *L. cephalotes, F. bengalensis, M. insignis, M. orientalis, S. japonica, S. chirayita, P. guajava, T. cordifolia* and *F. decipiens* showed a significant activity and *L. cephalotes, F. bengalensis, M. orientalis, S. japonica, P. guajava,* and *F. decipiens* were more potent than positive control. We had already reported hypoglycemic activity of *M. insignis, M. orientalis, S. japonica* and *S. chirayita.*[3,6] *Carthamus tinctorius* and *Zea mays* are not listed in Table I since they did not show any effect. One of the most active drugs mentioned above, *P. guajava* was fractionated and studied in detail which is discussed in the present paper.

P. guajava is a popular fruit in tropical region and the barks and leaves have been used for the treatment of diabetes traditionally in India and Nepal. There are some reports with regard to the isolation of alkaloids from this plant.[12] The sprout of this plant is a common herbal tea, specially drunk by the people of southern Japan and China. There is also a report on hypoglycemic activity of 50 % alcoholic extract of this plant on alloxan-induced diabetic rats.[12] In our study, alcoholic extract did not show any significant activity in STZ-induced diabetic rats, and on increasing the dose to 200 mg/kg, *i.p.* the drug was found to be toxic, although aqueous alcoholic extract showed a mild effect, while water extract was found to be highly effective to lower the blood glucose level. In order to confirm it, the sprout of this plant was extracted by four different methods ; a) by refluxing with 70 % ethanol, b) by extracting with water at 60°C, c) by extracting with ethanol at room temperature and d) by refluxing with water. Hypoglycemic activity of each extract was examined by administering 5 times of a dose of 100 mg/kg, *i.p.*, twice a day, and the results are shown in Fig. 1. Both water extracts, (extracted at 60°C and refluxed) lowered blood glucose level in STZ-induced diabetic rats by 54.26 and 57.1 %, respectively. In contrast, both alcoholic extracts did not show any significant effect.

Next, hypoglycemic activity of water extract (60 °C) of *P. guajava* was studied by oral administration. Only the groups given 200 and 100 mg/kg were found to be significantly different from control (Table II).

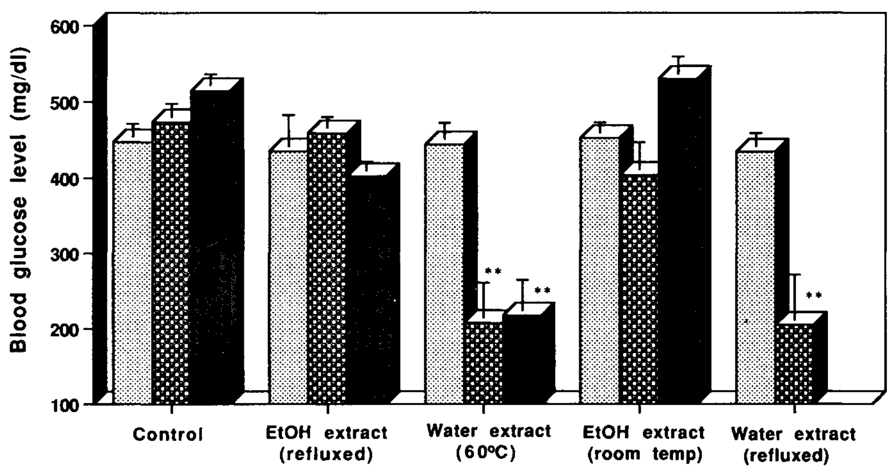

Fig. 1 Effects of different extracts of *Psidium guajava* (100 mg/kg, *i.p.*, twice a day) on blood glucose level in STZ-induced diabetic rats. Results are expressed as mean ± S.E., n= 6. Significantly different from control, $^{**}p<0.01$. ▒ Before *i.p.* ▩ After *i.p.* (6 h) ■ After *i.p.* (24 h)

Table II Effect of water extract of *Psidium guajava* (*p.o.*) on blood glucose level in STZ-induced diabetic rats.

Group	Dose (mg/kg)	Glucose level (mg/dl) before *p.o.*[a]	after *p.o.* (6 h)	after *p.o.* (24 h)	Decrease (%)[b] after 6 h	after 24 h
Control	···	442.0±40.0	505.0±18.5	509.8±8.2	···	···
I	200	443.8±37.9	424.2±27.7	450.5±31.5	16.2*	11.8
II	100	447.2±38.7	434.4±19.9	497.0±16.4	14.9*	3.6
III	50	436.6±31.3	458.4±38.0	468.8±40.0	8.1	6.8

Results are expressed as mean±S.E. n=5. Significantly different from control value, *$p<0.05$ a) Glucose level before administering the drugs or saline. b) Decrease in blood glucose level relative to that before *p.o.* administration, expressed in % compared to control. Groups I, II and III are water extract of *Psidium guajava* treated groups. Each group was treated five times, twice a day, with a dose listed in the table.

This result suggested that the drug was less effective on oral administration when compared with that of *i.p.* administration.

Under similar experimental conditions, the hypoglycemic activities of sprout and leaves were also compared. The extract of leaves lowered blood glucose level by 71.9 % while the extract of sprout lowered blood glucose level by 55.2 % (Fig. 2). This result clearly showed that the hypoglycemic activity of leaves was stronger than that of sprout.

Water extract of *P. guajava* obtained at 60°C was dialyzed through cellophane membrane MW 50,000) and it was separated into two fractions, PGW>50 and PGW<50. Both fractions were tested for their hypoglycemic activity. In this experiment also the drug was administered 5 times with a dose of 25 mg/kg, twice a day, *i.p.* and blood samples were analyzed 6 h and 24 h after the last dose administration. Both fractions showed a significant effect but PGW>50 was more active than PGW<50 with a dose of 25 mg/kg (Fig. 3).

In the next experiment, water extracts of sprout and leaves were fractionated into PGW 100 by ultrafiltration. Both PGW 100 of sprout and leaves showed hypoglycemic activity in STZ-induced diabetic rats (Fig. 4) and that of leaves was found to be more potent than that of sprout. However, when the activity of PGW 100 from leaves (Fig. 4) was compared with the activity of PGW>50 (Fig. 3), it was clear that PGW>50 was more effective, so the active com-

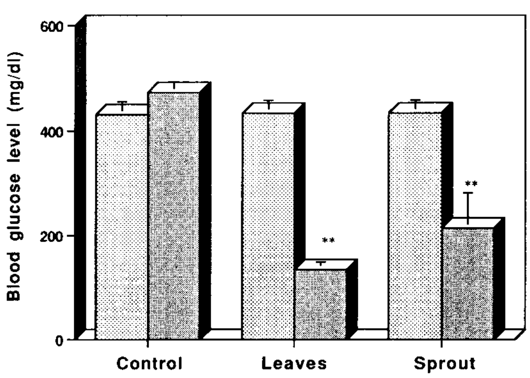

Fig. 2 Effects of extracts from leaves and sprout of *Psidium guajava* (5 times, 100 mg/kg, *i.p.*, twice a day) on blood glucose level in STZ-induced diabetic rats. Results are expressed as mean±S.E. of six experiments (n=6). Significantly different from control, **$p<0.01$. ▨ Before drug administration ▩ After drug administration

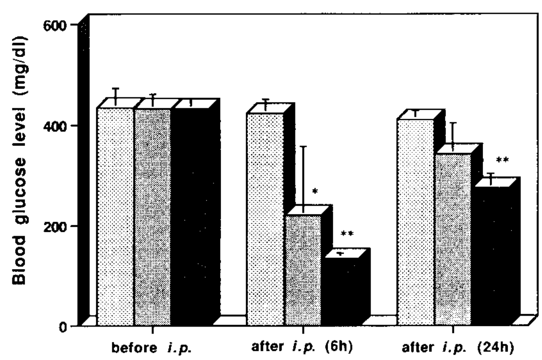

Fig. 3 Effects of fractions (PGW<50 and PGW>50) of *Psidium guajava* (water extract) obtained by the dialysis (each 5 times, 25 mg/kg, *i.p.*, twice a day) on blood glucose level in STZ-induced diabetic rats. Results are expressed as mean ± S.E., n=5. Significantly different from control, **$p<0.01$, *$p<0.05$.
▨ Control ▩ PGW<50 ■ PGW>50

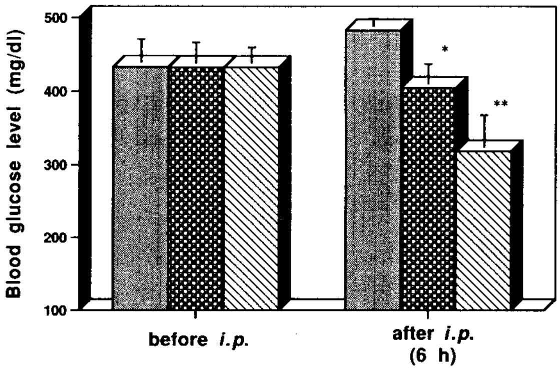

Fig. 4 Effects of water extract of *Psidium guajava* (sprout and leaves) with molecular weight larger than 100,000 (5 times, 25 mg/kg, twice a day, *i.p.*) on blood glucose level in STZ-induced diabetic rats. Results are expressed as mean±S.E., n=5, Significantly different from control, $**p<0.01$, $*p<0.05$. Control Sprout Leaves

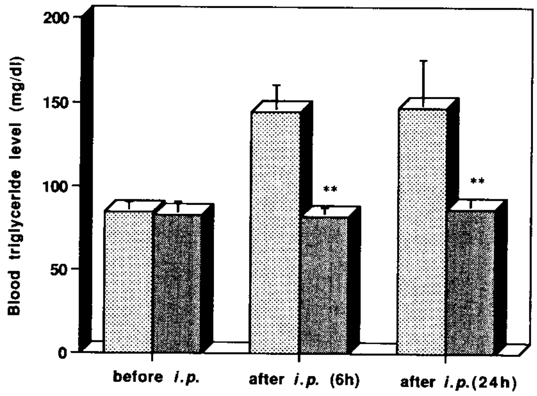

Fig. 5 Effects of water extracts (5 times, 100 mg/kg, *i.p.*, twice a day) of *Psidium guajava* on blood triglyceride level in STZ-induced diabetic rats. Results are expressed as mean±S.E., n=5, $**p<0.01$. Control Water extract

ponents which are responsible for lowering blood glucose level must have the molecular size between 50,000 and 100,000. Lowry's and phenol-H_2SO_4 tests suggested that the active fraction consists of glycoproteins.

We also examined the effect of water extract of *P. guajava* on blood triglyceride levels in STZ-induced diabetic rats. On administering 5 times with a dose of 100 mg/kg, *i.p.*, twice a day, triglyceride levels of water extract treated group were decreased by 35 % (Fig. 5) comparing with control and we observed no effect on cholesterol levels (data are not shown here) under similar experimental conditions.

Insulin assay : The immunoreactive plasma insulin level in PGW>50 treated groups was measured. After treating 5 times with a dose of 50 mg/kg, *i.p.*, insulin level of PGW>50 treated group, was 18.8±1.2 ng/dl. Comparing with control (17.3±2.0 ng/dl), there was no significant difference in insulin level.

2-DOG uptake activity : Glucose uptake activity was studied on Rat 1 fibroblast cells expressing human insulin receptor (HIRc-B).[9] The results of the

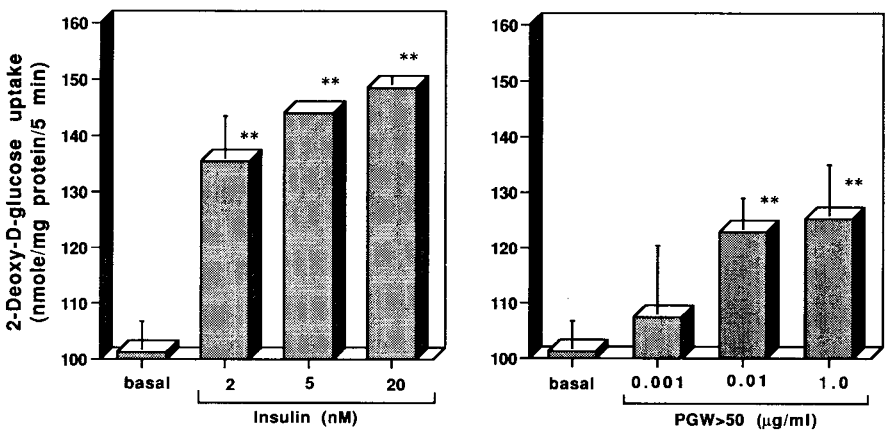

Fig. 6 2-Deoxy-D-glucose uptake activity stimulated by insulin (2, 5 and 20 nM) and PGW >50 (0.001, 0.01 and 1.0 μg/ml) in Rat 1 fibroblasts. Results are expressed as mean±S.E. of two experiments, n=3 in each case. Significantly different from control (basal), $**p<0.01$.

comparative 2-DOG uptake activity, stimulated by insulin and PGW>50 are shown in Fig. 6. The 2-DOG uptake activity of fibroblast cells was significantly stimulated by insulin at the concentration of 2, 5 and 20 nM. In the similar way PGW>50 also significantly stimulated the 2-DOG uptake activity by 21.37 % and 23.77 % in the concentrations of 0.01 and 0.1 μg/ml, respectively. When, however, PGW>50 (0.01 μg/ml) was mixed with 2 nM insulin, the effect was not stronger than that of the 2 nM insulin alone, namely PGW>50 did not have any additive effect to insulin.

The concentration of PGW>50 used in *in vitro* experiment that significantly stimulated 2-DOG uptake activity in fibroblast cells was the expected concentration in the blood of rats given *i.p.*, 25 mg/kg of PGW>50. The mechanism of hypoglycemic activity of PGW>50 is still unclear. Since we could not show any significant insulin increase in PGW > 50 treated diabetic rats, the drug might directly act as a hypoglycemic agent on peripheral tissues by a similar mechanism as vanadate[13,14] or it may have the extrapancreatic action like sulfonylurea.[15,16] The fact that this agent alone stimulated *in vitro* glucose uptake could support this hypothesis.

Conclusion

Twenty-three natural drugs were screened and nine of them showed a significant hypoglycemic activity in STZ-induced diabetic rats. The leaves of *P. guajava* were extracted by four different methods and only the water extracts showed a strong activity. Furthermore, we studied the activity of water extracts from sprout and leaves of *P. guajava* and both showed a strong activity but water extract of leaves was comparatively more effective than that of sprout. The results of the experiment suggested that the active component was a glycoprotein with the molecular size of 50,000 to 100,000. The drug was found to be more effective on administering *i.p.* than *p.o.* The water extract also reduced the blood triglyceride level. This drug did not improve insulin level, but was found to have 2-DOG uptake stimulating activity in the Rat 1 fibroblasts. *P. guajava* is a common herbal tea and present study suggested that it is very beneficial to lower the blood glucose level for diabetic patient or the aged people.

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和文抄録

伝統薬物について，STZ 誘発高血糖ラットを用いて血糖降下作用を検討した。その中で，9 種の薬物 *Ficus bengalensis, Filicium decipiens, Leucas cephalotes, Matteuccia orientalis, Morus insignis, Psidium guajava, Swertia japonica, S. chirayita* および *Tinospora cordifolia* が有意に血糖を降下することが判明した。特にグアバの水エキスは，STZ 誘発高血糖ラットに対して強い血糖降下作用が認められた。さらに，水エキスは透析膜および限外濾過によって分子量別に分別した。これらの分画の血糖降下作用の実験では，活性成分は 5 万から 10 万の分子サイズの糖蛋白であることが示唆された。また，グアバ葉の活性の方が新芽よりも活性が強いことが分かった。グアバ葉の水エキスでは，また，血中トリグリセライドレベルも有意に低下させた。さらに，分子量 5 万以上の分画は有意にかつ用量依存的にヒトインスリンレセプター発現ラット 1 フィブロブラストによる 2-deoxy-D-glucose の取り込み活性が認められた。

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