Identification of Five Phytosterols from Aloe Vera Gel as Anti-diabetic Compounds

Miyuki TANAKA,*[,a] Eriko MISAWA,[a] Yousuke ITO,[a] Noriko HABARA,[a] Kouji NOMAGUCHI,[a] Muneo YAMADA,[a] Tomohiro TOIDA,[a] Hirotoshi HAYASAWA,[a] Mitunori TAKASE,[a] Masanori INAGAKI,[c] and Ryuuichi HIGUCHI[c]

[a] *Biochemical Research Laboratory, Morinaga Milk Industry Co., Ltd.; 5–1–83 Higashihara, Zama, Kanagawa 228–8583, Japan: and* [b] *Faculty of Pharmaceutical Sciences, Kyushu University; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan.* Received February 20, 2006; accepted April 7, 2006; published online April 11, 2006

The genus *Aloe* in the family Liliaceae is a group of plants including Aloe vera (*Aloe barbadensis* MILLER) and *Aloe arborescens* (*Aloe arborescens* MILLER var. *natalensis* BERGER) that are empirically known to have various medical efficacies. In the present study, we evaluated the anti-hyperglycemic effect of Aloe vera gel and isolated a number of compounds from the gel. On the basis of spectroscopic data, these compounds were identified as lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol. These five phytosterols were evaluated for their anti-hyperglycemic effects in type 2 diabetic BKS.Cg-m$^{+/+}$Lepr$^{db/J}$ (*db*/*db*) mice. In comparison with the hemoglobin A1c (HbA1c) levels of vehicle-treated mice, statistically significant decreases of 15 to 18% in HbA1c levels were observed in mice treated with 1 μg of the five phytosterols. Considering the ability to reduce blood glucose *in vivo*, there were no differences between the five phytosterols. Administration of β-sitosterol did not reduce the blood glucose levels in *db*/*db* mice. After administration of the five phytosterols for 28 d, fasting blood glucose levels decreased to approximately 64%, 28%, 47%, 51%, and 55% of control levels, respectively. Severe diabetic mice treated with phytosterols derived from Aloe vera gel did not suffer weight reduction due to glucose loss in the urine. These findings suggest that Aloe vera gel and phytosterols derived from Aloe vera gel have a long-term blood glucose level control effect and would be useful for the treatment of type 2 diabetes mellitus.

Key words Aloe vera gel; type 2 diabetes; phytosterol

Of the more than 360 *Aloe* species known, *Aloe barbadensis* MILLER (Aloe vera) is the most widely used. Aloe vera contain two major parts: firstly, leaves containing a high concentration of anthraquinone compounds that have been used throughout the centuries as a cathartic and for medicinal purges; and secondly, a clear gel that has been used as a food and to treat burns and other wounds.[1,2]

Several chemical components of the *Aloe* gel are thought to be responsible for its wound healing and immunostimulatory properties. For example, glycoprotein Aloectin A is reported to have anti-tumor and anti-ulcer effects,[3] and a 29 kDa glycoprotein has been found to increase proliferation of normal human dermal cells.[4] Most of these polysaccharides are glucomannans, mannans, or pectins with a range of molecular weights. A major focus of research has been on the carbohydrate fraction isolated from *Aloe* gel known as "acemannan," which comprises a polydispersed β-(1,4)-linked acetylated mannan interspersed with *O*-acetyl groups.[5]

As a result of these studies, there have been numerous reports of *Aloe* having diverse biological activities, including anti-tumor activity, anti-acid activity,[6] tyrosinase inhibiting activity,[7] and antioxidant activity.[8]

Two clinical trials are available from the same research group that reported hyperglycemic effects on fasting blood glucose as well as on HbA1c levels[9,10] with Aloe vera gel. Hikino *et al.* isolated two hyperglycemic polysaccharides from *Aloe arborescens* at 1985.[11] Beppu *et al.* separated two different anti-diabetic components from the leaf pulp and leaf skin of the same plant. A previous study made with *Kidachi Aloe* (*Aloe arborescens* var. *naralensis*) in streptozotocin (STZ)-induced diabetic rats confirmed its efficacy through administration,[12] contrary to Koo who reported hyperglycemic effects in diabetic rats in acute phase with a product containing Aloe vera gel.[13]

In this study, we sought to determine the constituents of Aloe vera gel extract that normalize hyperglycemia in the diabetic mouse strain BKS.Cg-m$^{+/+}$Lepr$^{db/J}$ (*db*/*db*), which exhibits many of the metabolic disturbances of human type 2 diabetes including hyperglycemia, obesity, and insulin resistance. Hemoglobin A1c (HbA1c), a binding product of glucose and hemoglobin, increases depending on the severity of hyperglycemia in a glucose level-dependent manner, and the level of HbA1c reflects the past blood glucose control conditions over a long period. Therefore, we tried to isolate the bioactive compounds from Aloe vera gel based on their ability to decrease the HbA1c level of *db*/*db* mice. Finally, we examined whether phytosterols and fractions isolated from Aloe vera gel play an important role in anti-hyperglycemic activity.

MATERIALS AND METHODS

General Procedure Melting points were determined on a micro melting point apparatus (Yanako MP-3) without correction. NMR spectra were recorded using a Varian Unity-500 spectrometer (^{13}C: 125 MHz). Positive APCI-MS was taken with LC-MS 2000 (Shimadzu).

Preparation of Extracts Fresh leaf gels of Aloe vera (1 kg) were extracted three times with $CHCl_3$/MeOH (1 l each) at room temperature for 1 h, and separated into a $CHCl_3$/MeOH soluble fraction (T1 extract, 0.5 g) and an H_2O layer. The H_2O layer was further extracted three times with *n*-BuOH (1 l each) at room temperature for 3 h, and separated into a *n*-BuOH soluble fraction (T2 extract, 0.5 g) and an

* To whom correspondence should be addressed. e-mail: m_tanaka@morinagamilk.co.jp

H_2O layer. The H_2O layer was concentrated under reduced pressure followed by lyophilization to give the T3 extract (2.4 g). The T3 extract (2 g) was extracted three times with MeOH (200 ml each) at room temperature for 2 h, and separated into a MeOH layer and an insoluble mass (T5 extract, 14 g). Evaporation of the combined MeOH solution afforded the T4 extract (2.5 g).

Preparation of Compounds Dried Aloe vera (21 kg) was extracted three times with $CHCl_3$/MeOH (90 l). The $CHCl_3$/MeOH fraction (784 g) was subjected to silica gel column chromatography eluted with a gradient system of $CHCl_3$/MeOH (10 : 1→1 : 10) to provide 5 fractions. Fraction 1 (132.7 g) was further purified by silica gel column chromatography eluted with a gradient system of AcOEt/*n*-hexane (1 : 20→1 : 10) to provide 6 fractions (B1—B6). Fraction B1 (4 g) was further purified on a SP-120-40/60-ODS-A column eluted with MeOH/MeCN/THF/H_2O (76.5 : 10 : 9 : 4.5) to provide crude B11 and B12 fractions. The crude B11 and B12 fractions were isolated on an Inertsil ODS-3 column eluted with MeOH/MeCN/THF/H_2O (15 : 2 : 2 : 1) and purified by chromatography on a silica gel column using AcOEt/*n*-hexane (1 : 10) as the eluent to give purified compounds of B11 (228 mg) and B12 (197 mg).

B11: Amorphous powder, mp 100—101 °C. Positive APCI-MS exhibited *m*/*z* 411 $[M+H-H_2O]^+$, 429 $[M+H]^+$. The structure of B11 was determined as cycloartanol on the basis of ^{13}C-NMR spectral data by comparison with literature values.[14)]

B12: Amorphous powder, mp 118—120 °C. Positive APCI-MS exhibited *m*/*z* 423 $[M+H-H_2O]^+$. The structure of B12 was determined as 24-methylene-cycloartanol on the basis of ^{13}C-NMR spectral data by comparison with literature values.[14)]

The crude B2 fraction was purified by chromatography on a silica gel column using AcOEt/*n*-hexane (1 : 10) as the eluent to give purified compounds of B2 (80.5 g). B2 was identified by direct comparison (^{13}C-NMR) with an authentic sample of β-sitosterol.

Fraction B3 was mixed with dry-pyridine (35 ml), DMAP (12.3 mg), and acetic anhydride (10 ml) and reacted for 15 h at room temperature. The reaction mixture was diluted with H_2O, extracted with $CHCl_3$, and the solvent was evaporated. An acetyl derivative of fraction B3 (257 g) was purified by SP-120-40/60-ODS-A column chromatography and eluted with MeOH/MeCN/THF/H_2O (17 : 2 : 2 : 1) to provide three crude compounds (acetyl-B31, acetyl-B32 and, acetyl-B33). These crude compounds were further purified by Inertsil ODS-A column chromatography and eluted with MeOH/MeCN/THF/H_2O (16 : 2 : 2 : 1). The acetyl derivatives of the three compounds were added to a mixture of THF (150 ml), MeOH (300 ml), H_2O (100 ml), and K_2CO_3 (1 g). Each mixture was heated to 50 °C for 18 h and then extracted with $CHCl_3$ to isolate compounds B31 (89 mg), B32 (125 mg), and B33 (4 mg), respectively.

B31: Amorphous powder, mp 151—152 °C. Positive APCI-MS exhibited *m*/*z* 383 $[M+H-H_2O]^+$. The structure of B31 was determined as lophenol on the basis of ^{13}C-NMR spectral data by comparison with literature values.[14)]

B32: Amorphous powder, mp 174—175 °C. Positive APCI-MS exhibited *m*/*z* 397 $[M+H-H_2O]^+$. The structure of B32 was determined as 24-methyl-lophenol on the basis of ^{13}C-NMR spectral data by comparison with literature values.[14)]

B33: Amorphous powder, mp 168—169 °C. Positive APCI-MS exhibited *m*/*z* 411 $[M+H-H_2O]^+$. The structure of B33 was determined as 24-ethyl-lophenol on the basis of ^{13}C-NMR spectral data by comparison with literature values.[14)]

Animals and Treatment Fresh Aloe vera gel was cut into small pieces and homogenized with PBS in a blender. The final concentration of Aloe vera gel was adjusted to either 20, 30, or 50 mg/ml. Extracts and compounds were dissolved in DMSO (Sigma), and the concentration of each compound, extract, or fraction was adjusted to 1 μg/ml or 25 μg/ml with saline, respectively. The final concentration of DMSO was adjusted to 0.1%. Saline containing 0.1% DMSO was used as the vehicle. As a type 2 diabetes model, 6-week old male BKS.Cg-m$^{+/+}$Lepr$^{db/J}$ (*db*/*db*) mice were obtained from Charles River Japan (Tokyo, Japan). The mice were divided into several groups each consisting of 7 mice, and administered orally with vehicle as a control solution, 1 μg/mouse/d of a compound, or 25 μg/mouse/d of an extract or fraction.

Measurement of Blood Glucose Fasting blood glucose levels and random blood glucose levels were measured by using an Antsense analyzer (Bayer-Sankyo, Tokyo, Japan). The fasting blood glucose levels were measured after 15 h of fasting. HbA1C levels were measured using a DCA2000 analyzer (Bayer-Sankyo).

Immunostaining of Islets Pancreases from *db*/*db* mice treated with Aloe vela gel or vehicle were excised, fixed by immersion in 4% buffed formaldehyde, and then embedded in paraffin. Paraffin sections were incubated for 10 min with 3% H_2O_2 solution to block endogenous peroxidase activity and then overnight at 4 °C with guinea pig anti-insulin antibody (Dako Diagnostics, Missisauga, CA, U.S.A.). Sections were then incubated for 1 h with biotinylated anti-guinea pig antibody (Vector Laboratories, Berlingame, CA, U.S.A.), subsequently treated for 30 min with an avidin/biotin complex (Vectastain ABC kit, Vector Laboratories, Berlingame, CA, U.S.A.), and positive reactions were visualized by incubation with a peroxidase substrate solution containing 3,3′-diaminobenzidine tetrahydrochloride.

Statistics Data is presented as the mean±S.D. Statistical significance was assessed by group comparison with the use of one-way ANOVA followed by Tukey–Kramer test. Significance was accepted at $p<0.05$.

RESULTS

Previous reports demonstrated that Aloe vera gel extract has a protective effect comparable to glibenclamide against hepatotoxicity in neonatal streptozotocin (n0STZ)-induced

Table 1. Fasting Blood Glucose Levels of *db*/*db* Mice Treated with Aloe Vera Gel or Saline

		Fasting blood glucose levels (mg/dl)		
		Day 0	Day 15	Day 29
Saline		74.0±36.3	304.0±173.8	463.0±89.5
Aloe vera gel	20 mg	69.8±14.2	268.4±94.5	335.2±93.4*
	30 mg	66.0±5.4	177.3±62.7	363.3±64.5*
	50 mg	79.7±19.1	166.5±26.2	277.7±60.8**

Seven *db*/*db* mice each were daily administered orally with saline or the indicated dose of Aloe vera gel and fasting blood glucose was measured on Day 15 and Day 29. Significantly different from saline-injected mice (* $p<0.05$ and ** $p<0.005$).

type-II diabetic rats.[14] Okyar *et al.* reported that Aloe vera gel extract showed hyperglycemic activity in NIDDM (Non-Insulin Dependent Diabetes Mellitus) rats.[15] Therefore, we tried to administer type 2 diabetes model mice with Aloe vera gel. After administration for 15 d, fasting blood glucose levels of mice treated with 10, 20, or 30 mg/mouse/d of Aloe vera gel were reduced to 88%, 58%, and 54% respectively, of the control level (Table 1). On day 29, fasting blood glucose levels of mice treated with Aloe vera gel were decreased significantly compared to controls ($p<0.05$ and $p<0.005$). On day 35, when pancreas tissue was stained with anti-insulin antibodies, those of *db*/*db* mice treated with 50 mg/mouse/d of Aloe vera gel exhibited strong staining (Fig. 1B), as compared with the pancreas tissue of control littermates (Fig.

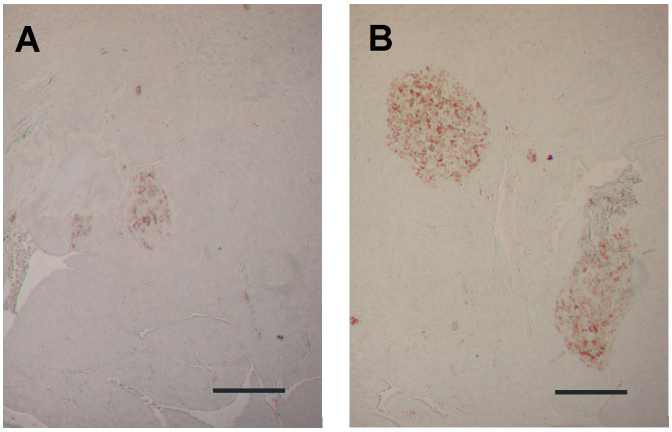

Fig. 1. Immunostaining for Insulin in Pancreas of *db*/*db* Mice

In saline-treated mice (A), islets were irregular in shape and weak immunostaining was observed. In contrast, islets from mice treated with 50 mg/mouse/d of Aloe vera gel (B) showed intense insulin immunostaining. Bar, 200 μm.

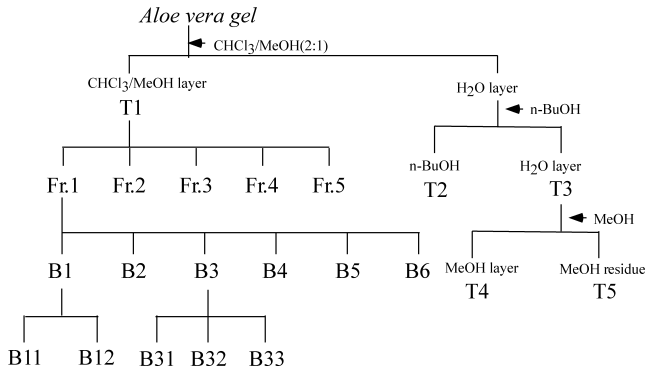

Fig. 2. Isolation Method for Anti-hyperglycemic Compounds from Aloe Vera Gel

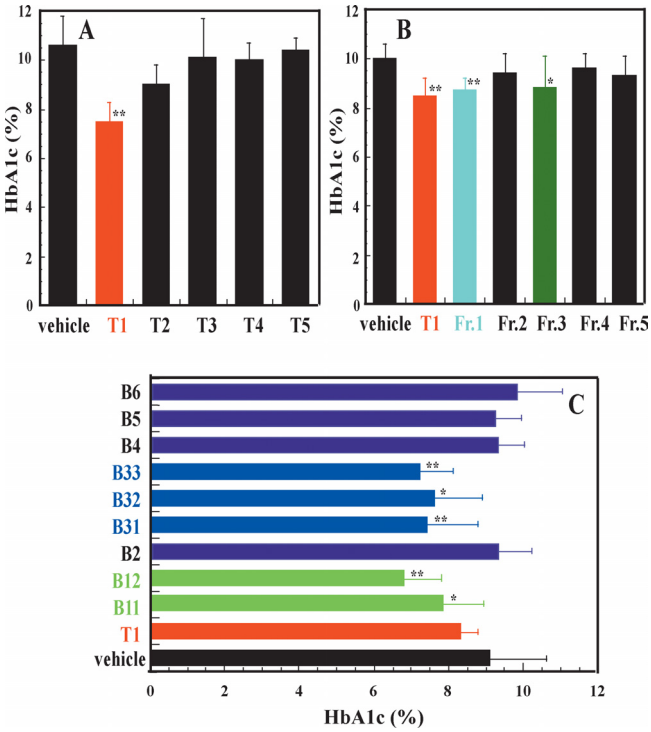

Fig. 3. The *in Vivo* Effects of Compounds Derived from Aloe Vera Gel on HbA1c Levels

Seven *db*/*db* mice each were daily administered orally with 0.1% DMSO as the control solution or 1 μg of compound or 25 μg/mouse/d of extract/fraction. Significantly different from vehicle-injected mice ($*p<0.05$ and $**p<0.005$).

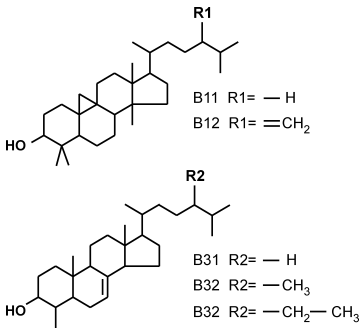

Fig. 4. Chemical Structures of Compounds B11, B12, B31, B32, and B33

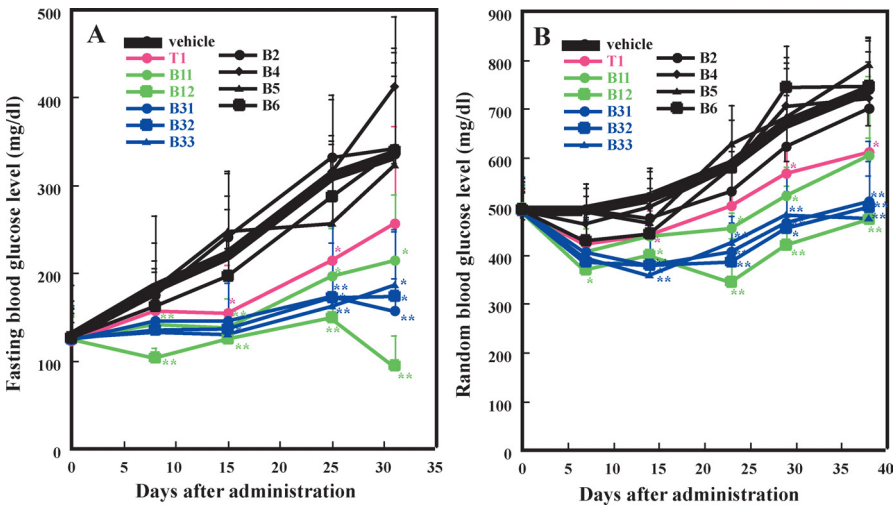

Fig. 5. Phytosterols Derived from Aloe Vera Gel Prevented the Development of Diabetes in *db*/*db* Mice

Seven *db*/*db* mice each were daily administered orally with 0.1% DMSO as the control solution or 1 μg/mouse/d of B11, B12, B2, B31, B32, B33, or 25 μg/mouse/d of B4, B5, B6, or T1. Significantly different from vehicle-injected mice ($*p<0.05$ and $**p<0.005$).

1A). The size of islets derived from saline-treated mice appeared smaller than those derived from mice treated with Aloe vera gel. The number of islets derived from mice treated with 20 mg/mouse/d of Aloe vera gel for 35 d was 1.7-fold higher than from saline-treated mice (data not shown).

Fresh Aloe vera gel was homogenized with $CHCl_3$/MeOH (2 : 1) solution in a mixer and divided into $CHCl_3$/MeOH and H_2O layers. Part of the H_2O layer was successively divided into a *n*-BuOH extract or a MeOH layer. Finally, five extracts were separated from Aloe vera gel by the procedures shown in Fig. 2, and were separately administered to *db*/*db* mice. When evaluated after the administration of T1 extract (25 μg/mouse/d) for 12 d, the fasting blood glucose level was significantly reduced to 85% of the controls, but the other four extracts did not exhibit the ability to reduce the blood glucose level of *db*/*db* mice (data not shown). After 35 d, administration of T1 extracts (25 μg/mouse/d) caused a reduction in HbA1c levels (Fig. 3A). Therefore, anti-diabetic compounds were found to be concentrated in the T1 extract. The T1 fraction was subjected to normal-phase silica gel column chromatography and was divided into 5 fractions (F1—F5). After 29 d, the HbA1c levels of *db*/*db* mice administered with 25 μg/mouse/d of F1 or F3 were 1.3% and 1.2%, respectively, lower than mice administered with vehicle (Fig. 3B).

We attempted to isolate the effective compounds from F1. F1 was subjected to normal-phase silica gel column chromatography and divided into six fractions (B1—B6). Fractions B1, B2, and B3 were further purified by chromatography to give six compounds. As shown in Fig. 4, the structures of the six compounds were identified as phytosterols by detailed ^{13}C-NMR spectroscopy and by comparison of the spectral data with that of published values.[16]

We next examined whether *in vivo* treatment with phytosterols and other fractions decreased the blood glucose levels of *db*/*db* mice. The results of HbA1c level measurements on the 35th day from the start of administration with 1 μg/mouse/d of B11, B12, B2, B31, B32, or B33 and 25 μg/mouse/d of B4, B5, B6, or T1 are shown in Fig. 3C. In comparison with the HbA1c levels of mice treated with vehicle a statistically significant decrease of 15 to 18% was observed in mice treated with 1 μg/mouse/d of the five phytosterols derived from fraction B1 or fraction B3. The decreases caused by other fractions or B2 (β-sitosterol) were found to be insignificant.

Blood glucose levels of *db*/*db* mice treated chronically with the five phyotsterols are shown in Fig. 5. In serious diabetic model mice, 1 μg/mouse/d of chronic phytosterols derived from Aloe vera gel resulted in a decrease in both levels of fasting blood glucose and random blood glucose compared to vehicle controls, reaching statistical significance from Day 4 or Day 5 (Figs. 5A, B). After administration for 28 d, fasting blood glucose levels were decreased to approximately 64%, 28%, 47%, 51%, and 55% of the control levels, by administration of 1 μg/mouse/d of B11, B12, B31, B32, and B33, respectively. In comparison with the blood glucose levels of mice treated with vehicle, there was no difference in the blood glucose levels of mice treated by B2 (β-sitosterol) administration.

We observed a decrease of body weight in vehicle-treated mice, but not in mice treated with Aloe vera gel or its components (Table 2) over the course of the experiment. While weights of the groups were similar on Day 0, following the 33 d of the experiment, the vehicle-treated mice weighted on average approximately 2.9 to 6.4 g less than the mice treated with Aloe vera gel or its components.

Table 2. Preventative Effects of Phytosterols Derived from Aloe Vera Gel on Body Weight Loss in Severe Diabetic Mice

		Body weight (g)	
		Day 0	Day 33
Vehicle	—	36.5±2.4	34.7±3.6
Aloe vera gel	50 mg	36.9±1.1	37.6±3.9
T1 extract	25 μg	36.8±0.7	39.6±2.0*
B11	1 μg	36.9±1.5	38.7±2.7
B12	1 μg	36.8±2.1	41.1±2.0*
B31	1 μg	37.2±0.8	39.2±2.4*
B32	1 μg	37.1±1.2	39.0±3.4
B33	1 μg	36.9±1.5	39.7±5.6

Seven *db*/*db* mice each were daily administered orally with 0.1% DMSO as the control solution or 1 μg/mouse/d of compound or 25 μg/mouse/d of T1 extract. Significantly different from vehicle-injected mice (* $p<0.05$).

DISCUSSION

Previous studies demonstrated that an alcoholic extract of Aloe vera gel maintained the glucose homeostasis of streptozotocin-induced diabetic rats by controlling the carbohydrate metabolizing enzymes.[17] However, little is known about the structures of the active compounds in Aloe vera gel. The present study, in which we identified five minor phytosterols, should help us to comprehend the anti-diabetic mechanisms of Aloe vera gel.

β-Sitosterol, campesterol, and stigmasterols are abundant plant sterols and are structurally similar to cholesterol. It was recognized in the 1950s that plant sterols lower serum concentrations of cholesterol. They do this by reducing the absorptions of cholesterol from the gut by competing for the limited space for cholesterol in mixed micelles.[18,19]

In this study, we observed that 1 μg of phytosterols derived from Aloe vera gel lower blood glucose levels; however, we did not observe the reduction of serum concentrations of cholesterols (data not shown). This may be simply explained by presuming that the effective dose that was applicable to decrease serum cholesterol levels was more than 100-fold higher than that applicable to decrease blood glucose levels.[20] It was also possible that the effective structure for the reduction of serum cholesterol was the 4-desmethyl moiety (containing no methyl groups at carbon atom 4), while the structures of anti-hyperglycemic phytosterols derived from Aloe vera gel were 4-monomethyl and 4-dimethyl sterols.

In considering structure, the anti-hyperglycemic phytosterols derived from Aloe vera gel fall into two groups of compounds, the lophenol group and the cycloartane group. Lophenol is known to be an intermediate of the biosynthetic pathway for squalane in plants[21]; however, the effect of this compound *in vivo* is unknown. A previous report suggested that compounds with a cycloartane structure *e.g.*, cycloartanol, had the ability to prevent cancer.[22] However, the effect

of cycloartane compounds on diabetes mellitus was unknown.

As shown in Fig. 3B, anti-hyperglycemic effects were also observed in fraction 3. We tried to isolate and purify the active compounds from fraction 3, and obtained two crude fractions. Because both compounds showed a *Rf* values very close to that of β-sitosterol glucoside in an examination based on TLC, it was anticipated to be a glycoside in which one molecule of sugar was bound to the aglycon moiety. To examine the sugar composition of the methanolysis product, it was made into a Trimethylsilyl (TMS) derivative and subjected to GC-MS measurement. The main peaks were substantially consistent with the main peaks of authentic glucose (data not shown). After the crude glycosides were methanolyzed and acetylated, these agflycons were isolated. The aglycon moieties coincided with 24-methyl-lophenol and 24-ethyl-lophenol by detailed ^{1}H- and ^{13}C-NMR spectroscopy (data not shown).

This study demonstrated that long-term treatment with Aloe vera gel ameliorates hyperglycemia in diabetic C57BL/KS-Lep^{db} (*db*/*db*) mice, where the mice lack functional leptin receptor.[23,24] In this strain of mice, metabolic abnormalities manifest early during development and are quite severe in young adult animals. Moreover, once established, these metabolic changes are resistant to modulation by caloric restriction or weight reduction compared with other mouse models of obesity-associated insulin resistance and dyslipidemia.[25] We observed weight reduction in vehicle-treated mice in line with previous reports (Table 2). In contrast, the mice treated with Aloe vera gel or its effective compounds did not experience weight reduction. This differential body weight response most likely reflects the more severe hyperglycemia of the vehicle-treated mice, which will lose more calories due to glucose loss in the urine.

Administration of phytosterols derived from Aloe vera gel did not change the blood glucose levels in a normal C57BL/6J mouse (data not shown). In addition, the pre-administration of phytosterols did not change the glucose tolerance of normal mice (data not shown).

It is well known that mice fed a high fat diet develop obesity and hyperglycemia and are used as a model of noninsulin-dependent diabetes mellitus.[26] We have evaluated the effects of Aloe vera gel in high fat diet-fed obese mice. Administration of Aloe vera gel with the high fat diet prevented the development of insulin resistance and glucose intolerance (data not shown). Further studies are required to determine the molecular basis of the effect of anti-diabetic phytosterols on the normalization of insulin resistance and glucose intolerance.

There was no case showing acute hypoglycemic conditions during the administration of Aloe vera gel or its anti-diabetic compounds, and no adverse side effect symptoms were observed from the viewpoints of pathological findings. Thus, the phytosterols derived from Aloe vera gel could be useful compounds for the treatment or preventor of type 2 diabetes mellitus.

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REFERENCES

1) Grindlay D., Reynolds T., *J. Ethnopharmcol.*, **16**, 117—151 (1986).
2) Joshi S. P., *J. Med. Aromat. Plant Sci.*, **20**, 768—773 (1998).
3) Imanishi K., *Phyother. Res.*, **7**, S20—S22 (1993).
4) Yagi A., Egusa T., Arase M., Tanabe M., Tsuji H., *Planta Med.*, **63**, 18—21 (1997).
5) Manna S., McAnalley B. H., *Carbohydr. Res.*, **241**, 317—319 (1993).
6) Hirata T., Suga T., Z. *Naturforsc.*, **32c**, 731—734 (1977).
7) Piao L. Z., Park H. R., Park Y. K., Lee S. K., Park J. H., Park M. K., *Chem. Pharm. Bull.*, **50**, 309—311 (2002).
8) Yagi A., Kabash A., Mizuno K., Moustafa S. M., Khalifa T. I., Tsuji H., *Planta Med.*, **69**, 269—271 (2003).
9) Yongchaiyudha S., Rungpitarangsi V., Bunyapraphatsara N., Chokechaijaroenporn O., *Phytomedicine*, **3**, 241—243 (1996).
10) Bunyapraphatsara N., Yongchaiyudha S., Rungpitarangsi V., Chokechaijaroenporn O., *Phytomedicine*, **3**, 245—248 (1996).
11) Hikino H., Takahashi M., Murakami M., Konno C., *Int. J. Crude Drug Res.*, **24**, 183—186 (1986).
12) Beppu H., Nagamura Y., Fujita K., *Phytother. Res.*, **7**, S37—S42 (1993).
13) Koo M. W. L., *Phytother. Res.*, **8**, 461—464 (1994).
14) Akihisa T., Matsumoto T., *Yukagaku*, **36**, 301—319 (1987).
15) Can A., Akev N., Ozsoy N., Bolkent S., Arda B. P., Yanardag R., Okyar A., *Biol. Pharm. Bull.*, **27**, 694—698 (2004).
16) Okyar A., Can A., Akev N., Baktir G., Sutlupinar N., *Phytother. Res.*, **15**, 157—161 (2001).
17) Akihisa T., Matsumoto T., *Jpn. Oil Chem. Soc.*, **36**, 301—319 (1987).
18) Rajasekaran S., Sivagnanam K., Ravi K., Subramanian S., *J. Med. Food*, **7**, 61—66 (2004).
19) Ikeda I., Tanaka K., Sugano M., Vahouny G. V., Gallo L. L., *J. Lipid. Res.*, **29**, 1573—1582 (1988).
20) Valkema A. J., *Acta Physiol. Pharmacol. Neerl.*, **4**, 291—292 (1955).
21) Uchida K., Mizuno H., Hirota K., Takeda K., Takeuchi N., Ishikawa Y., *Jpn. J. Pharmacol.*, **33**, 103—112 (1983).
22) Ikeda T., *Jpn. Oil Chem. Soc.*, **23**, 233 (1974).
23) Smith-Kielland I., Dornish J. M., Malterud K. E., Hvistendahl G., Romming C., Bockman O. C., Kolsaker P., Stenstrom Y., Nordal A., *Planta Med.*, **62**, 322—325 (1996).
24) Lee G. H., Proenca R., Montez J. M., Carroll K. M., Darvishzadeh J. G., Lee J. I., Friedman J. M., *Nature* (London), **379**, 632—635 (1996).
25) Chua S. C., Jr., Chung W. K., Wu-Peng X. S., Zhang Y., Liu S. M., Tartaglia L., Leibel R. L., *Science*, **271**, 994—996 (1996).
26) Tonra J. R., Ono M., Liu X., Garcia K., Jackson C., Yancopoulos G. D., Wiegand S. J., Wong V., *Diabetes*, **48**, 588—594 (1999).
27) Rossmeisl M., Rim J., Koza R., Kozak L., *Diabetes*, **52**, 1958—1966 (2003).