Administration of Dried Aloe vera Gel Powder Reduced Body Fat Mass in Diet-Induced Obesity (DIO) Rats

Eriko MISAWA, Miyuki TANAKA, Kazumi NABEISHIMA, Kouji NOMAGUCHI, Muneo YAMADA, Tomohiro TODA and Keiji IWATSUKI

Functional Food Research Department, Food Science & Technology Institute, Morinaga Milk Industry Co. Ltd., Higashihara 5–1–83, Zama, Kanagawa 252–8583, Japan

(Received October 17, 2011)

Summary The aim of the present study was to investigate the anti-obesity effects of Aloe vera gel administration in male Sprague-Dawley (SD) rats with diet-induced obesity (DIO). SD rats at 7 wk of age were fed either a standard diet (10 kcal% fat) (StdD) or high-fat (60 kcal% fat) diet (HFD) during the experimental period. Four weeks after of HFD-feeding, DIO rats (11 wk of age) were orally administered with two doses of Aloe vera gel powder (20 and 200 mg/kg/d) for 90 d. Body weights (g) and body fat (%) of HFD fed rats were significantly higher than those of StdD-fed rats. Although a modest decrease of body weight (g) was observed with the administration of dried Aloe vera gel powder, both subcutaneous and visceral fat weight (g) and body fat (%) were reduced significantly in Aloe vera gel-treated rats. Serum lipid parameters elevated by HFD were also improved by the Aloe vera gel treatment. The oxygen consumption (VO2), an index of energy expenditure, was decreased in HFD-fed rats compared with that in StdD-fed rats. Administration of Aloe vera gel reversed the change in VO2 in the HFD-fed rats. These results suggest that intake of Aloe vera gel reduced body fat accumulation, in part, by stimulation of energy expenditure. Aloe vera gel might be beneficial for the prevention and improvement of diet-induced obesity.

Key Words Aloe vera gel, diet-induced obesity, body fat, anti-obesity

The increasing prevalence of obesity is a serious public health problem throughout the world. Obesity is sufficient to cause all types of lifestyle-related chronic diseases, such as hypertension, hyperglycemia, dyslipidemia, and arteriosclerosis, so-called metabolic syndrome (1). From the accumulated evidence, it is considered that visceral obesity is the most crucial risk factor for metabolic syndrome. Therefore, preventing and improving obesity is important to reduce the risks of critical cardiovascular diseases induced by metabolic syndrome.

Aloe species have been used for their anti-tumor, antioxidant, anti-inflammatory, and laxative effects (2–4). Aloe barbadensis Miller (Aloe vera), a member of the family Liliaceae, is a traditional medicinal plant. From the inner gel of the Aloe vera leaf (the mesophyll part of Aloe vera), over 75 active ingredients, such as anthraquinones, carbohydrates, chromones, enzymes, inorganic compounds, lipids, sterols (lupeol, campesterol, and β-sitosterol), tannins, amino acids, proteins, saccharides, and vitamins, have been identified (5–8). The major components of Aloe vera gel are polysaccharides (pectins, hemicelluloses, glucomannan, acemannan, and mannose derivatives), and they have been well studied. Polysaccharides isolated from the gel of Aloe species have various biological activities, including immunomodulatory effects. For example, polysaccharides of between 400 Da and 5 kDa were reported to exhibit potent antitumor activity in vivo (9). Yagi et al. isolated a glycoprotein from Aloe that inhibited cyclooxygenase and reduced thromboxane synthesis (10).

The anti-diabetic activity of Aloe vera has been also demonstrated. Two nonrandomized clinical studies found that 2 tablespoons of Aloe vera gel juice a day significantly reduced fasting blood glucose levels in diabetic patients after 6 wk of intake (11, 12). Additionally, Aloe vera can be useful for reducing lipid levels in patients with hyperlipidemia (11). In animal studies, the hypoglycemic effects of Aloe vera gel on streptozotocin-induced diabetic models have been demonstrated (13, 14). Recently, Jain et al. reported the cardioprotective activity of Aloe vera gel treatment in streptozotocin-induced diabetic rats (15).

In our previous study, we confirmed the anti-hyperglycemic effect of Aloe vera gel and succeeded in identifying five kinds of phytosterols (lophenol, 24-methylene-cycloartanol) as anti-diabetic compounds (16). Additionally, we found that the administration of two Aloe-stereols (lophenol and cycloartanol) could improve hyperglycemia and glucose intolerance, and reduce visceral fat mass without weight loss in Zucker diabetic fatty (ZDF; ZDF/Crl-Lepr°(fa/fa)) rats (17). Furthermore, we demonstrated that these Aloe-stereols activated transcription of PPARs using a luciferase reporter assay (18).

In this study, we investigated the anti-obesity effects of Aloe vera gel on changes in body weight gain, food
intake, body fat mass, serum lipid profiles, and energy expenditure in diet-induced obesity (DIO) rats.

MATERIALS AND METHODS

Preparation of Aloe vera gel powder. Dried Aloe vera gel powder was obtained from Aloe vera gel, the inside portion of leaves, by hot-air drying. For the administration sample, the dried Aloe vera gel powder was suspended in distilled water and the dosage of homogenized suspension was adjusted to 4 and 40 mg/mL.

Animals. Six-week-old male Sprague-Dawley (SD) rats were purchased from Charles River Japan, Inc. (Kanagawa, Japan). Rats were housed under a 12-h light and dark cycle (lights 8:00 a.m.–8:00 p.m.) with free access to food and water. The use of animals in this study was in accordance with a protocol that was approved by the Morinaga Milk Industry Co., Ltd. Animal Care Committee.

Experimental protocol. After 1 wk of receiving a standard diet containing 10 kcal% fat (D12450B; Research Diets, New Brunswick, NJ, USA) for acclimatization, the rats were randomly divided into four groups. Treatment groups were defined as follows:
1) Standard diet group (StdD)
2) High-fat-diet control group (HFD-control)
3) Low-dose Aloe (20 mg/kg/d)-treated high-fat-diet group (HFD + Aloe 20)
4) High-dose Aloe (200 mg/kg/d)-treated high-fat-diet group (HFD + Aloe 200)

The standard diet group (StdD) was given the standard diet (D12450B) continuously and other groups were fed a high-fat diet (HFD) containing 60 kcal% fat (D12492; Research Diets) throughout the study. At 11 wk of age, the HFD-fed DIO rats were started on a program of administration of Aloe vera gel. They were orally administered 0.5 mL/100 g body weight of a suspension of Aloe vera gel powder (20 or 200 mg/kg/d) once a day every day for 90 consecutive days. As the HFD-control, the same volume of distilled water was administered. During the treatment period, body weights and food intakes were monitored.

At the end of the feeding period (24 wk of age), the rats were sacrificed without starvation and blood was collected by cardiac puncture. Serum was prepared by centrifugation of the blood at 1,000 × g for 10 min at 4°C and stored at −80°C until analysis. The livers and intra-abdominal adipose tissues (epididymal, mesenteric, and retroperitoneal fat pads) were excised and weighed.

Computed tomography (CT) analysis. CT-based analysis of body composition was performed after 12 wk of Aloe vera gel treatment (at 23 wk of age). The rats were anesthetized with isoflurane (Dainippon Sumitomo Pharma, Osaka, Japan) and scanned from xiphisternum to sacrum at 2 mm intervals using LaTheta (LCT-100A), an experimental X-ray CT instrument (Aloka Co., Ltd., Tokyo) (19). The estimated amounts of visceral and subcutaneous fat tissues, and percentage of body fat were calculated using LaTheta software.

Measurement of energy expenditure. On the 8th week from the start of the administration (at 19 wk of age), each rat was placed in an individual metabolic chamber and oxygen consumption (VO$_2$) and carbon dioxide production (VCO$_2$) were measured simultaneously for 4 animals using an indirect calorimetric system (MM202R: Muromachi Kikai Co., Ltd., Tokyo, Japan). After the rats had adapted to the environment of the metabolic chamber, whole-body oxygen consumption rate was measured every 5 min for 22 h. The respiratory quotient (RQ) was calculated as VCO$_2$/VO$_2$.

Biochemical analysis. Serum concentrations of triglyceride (TG), non-esterified fatty acid (NEFA), and total cholesterol (T-Chol) were determined using a commercial kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Statistical analysis. The results are presented as mean ± SE. Two-tailed Student’s t-tests were used to compare StdD-fed lean and obese HFD-control groups. Among HFD-fed DIO rats, the statistical differences were assessed by Dunnett’s multiple comparison tests. Differences were considered to be significant when p values were less than 0.05.
RESULTS

Growth curve and food intake

Figure 1A shows the change in body weight of SD rats during the experiments. On the initial day of the administration period (shown by an arrow in the figure), body weight of the HFD-control group was significantly higher than that in the StdD group, but there were no significant differences in body weight among the three DIO groups (fed with HFD). Body weight gain in the HFD-control was significantly higher than that of the StdD-fed rats throughout the experimental period. Both low- (Aloe 20) and high-dose (Aloe 200) groups tended to have slightly lower body weight than HFD-control after the start of Aloe vera gel treatment. At the end of the experiments, the incremental ratios of body weight gain during the treatment period were 63.8% (from 369.3 ± 5.1 g to 605.0 ± 16.2 g) in the StdD group, 68.7% (from 449.7 ± 12.3 g to 758.6 ± 31.3 g) in the HFD-control, 67.9% (from 443.2 ± 8.9 g to 744.0 ± 26.7 g) in the HFD+low-dose Aloe group, and 62.8% (from 441.1 ± 8.2 g to 714.1 ± 18.2 g) in the HFD+high-dose Aloe group (Table 1).

The food intake during the experiment was also measured. Feeding on HFD caused a marked decrease in food intake (g/d) compared with that when feeding on StdD, but there was no significant difference in caloric intake (kcal/d) between the HFD-control and StdD groups (Fig. 1B, Table 1). Additionally, Aloe vera gel treatment did not affect the food intake in the DIO rat groups (Fig. 1B, Table 1). Notably, no adverse effects following the administration of Aloe vera gel were observed.

Effects of Aloe vera gel on body fat mass and liver

To determine the body fat mass, we performed X-ray CT analysis. As shown in Fig. 2, the estimated amounts of both visceral (4.12-fold, p<0.001) and subcutaneous fat (4.71-fold, p<0.001) of HFD-control were significantly higher than in the StdD group. Intergroup comparison in the DIO rats showed that the amounts of both visceral and subcutaneous fats were clearly decreased in the Aloe vera gel-treated group compared with those in the HFD-control. In particular, in the rats with high-dose treatment of Aloe vera gel (HFD+Aloe 200), visceral and subcutaneous fat masses were 70.5% and 53.2% of those in HFD-control group, with significant reduction. As with the amount of body fat

Table 1. Effects of Aloe vera gel powder on body weight and food intake in male SD rats fed a high-fat-diet (HFD) or standard diet (StdD) for 12 wk.

<table>
<thead>
<tr>
<th></th>
<th>StdD</th>
<th>HFD-control</th>
<th>HFD+Aloe 20</th>
<th>HFD+Aloe 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>369.3±5.1</td>
<td>449.7±12.3***</td>
<td>443.2±8.9</td>
<td>441.1±8.2</td>
</tr>
<tr>
<td>Final</td>
<td>605.0±16.2</td>
<td>758.6±31.3***</td>
<td>744.0±26.7</td>
<td>714.1±18.2</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>235.7±13.8</td>
<td>297.2±20.8*</td>
<td>302.0±19.7</td>
<td>277.7±10.7</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>26.2±1.0</td>
<td>19.5±1.2***</td>
<td>19.0±0.9</td>
<td>19.1±0.8</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>100.9±3.5</td>
<td>102.2±6.6</td>
<td>99.6±4.6</td>
<td>100.1±4.7</td>
</tr>
</tbody>
</table>

Values are means±SE of seven animals.
Significantly different from the lean group at *p<0.05 and ***p<0.001, respectively.
mass, the body fat percentage was significantly increased (2.87-fold, \(p<0.001\)) by HFD feeding, and it also exhibited 18.0% reduction by treatment of Aloe vera gel at 200 mg/kg (\(p<0.05\) vs. HFD-control).

Then, we measured the actual weights of epididymal fat, mesenteric fat, and retroperitoneal fat in DIO rats with consecutive treatment of Aloe vera gel. Predictably, all intra-abdominal adipose tissue was significantly increased in DIO rats compared with that in the StdD group (Table 2). Consistent with the CT data (Fig. 2), the weights of mesenteric and retroperitoneal fat in the DIO rats treated with high-dose Aloe vera gel (HFD + Aloe 200) were lower than those of HFD-control rats, but the weights of epididymal fat were not changed. Compared with that in the HFD-control group, the summed weights of the three parts of the intra-abdominal fat pads were 3.9 and 18.9% lower in HFD + Aloe 20 and HFD + Aloe 200 groups, respectively.

Additionally, we also measured the weights of livers (Table 2). As with the body fat weights, the liver weights were significantly increased (1.25-fold, \(p<0.05\)) by HFD feeding. The treatment of high-dose Aloe vera gel (HFD + Aloe 200) reduced the liver weights significantly (\(p<0.05\)). However, the weights of livers in the DIO rats

### Table 2. Effects of Aloe vera gel powder on absolute weights of liver and intra-abdominal adipose tissues in male SD rats fed a high-fat-diet (HFD) or standard diet (StdD) at the age of 24 wk (on the 91st day from the start of the treatment).

<table>
<thead>
<tr>
<th></th>
<th>StdD</th>
<th>HFD-control</th>
<th>HFD + Aloe 20</th>
<th>HFD + Aloe 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (g)</td>
<td>18.1±0.3</td>
<td>22.7±1.5*</td>
<td>21.2±1.7</td>
<td>18.6±0.5*</td>
</tr>
<tr>
<td>Intra-abdominal fat (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epididymal</td>
<td>9.5±0.6</td>
<td>22.2±1.4**</td>
<td>22.5±7.7</td>
<td>21.4±2.8</td>
</tr>
<tr>
<td>Mesentrium</td>
<td>5.9±0.8</td>
<td>19.3±2.7###</td>
<td>16.8±1.8</td>
<td>13.5±2.6*</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>12.5±1.9</td>
<td>41.3±4.1###</td>
<td>40.3±5.2</td>
<td>32.8±2.3</td>
</tr>
</tbody>
</table>

Values are means±SE of seven animals (n=7).
Significantly different from the lean group at *\(p<0.05\) and **\(p<0.01\), respectively.
* Significantly different from the HFD-control group at \(p<0.05\).
treated with low-dose Aloe vera gel (HFD + Aloe 20) were decreased slightly.

**Effects of Aloe vera gel on serum lipid**

To clarify the effects of Aloe vera gel on lipid metabolism, we measured the serum TG, NEFA, and T-Cholesterol levels in the DIO rats after the consecutive treatments. Data for Fig. 3 indicate that all lipid parameters of DIO rats were higher than in the StdD group, but serum NEFA level was increased slightly by HFD. As shown in Fig. 3A, the serum TG levels were 32.6% and 40.7% lower than that of HFD-control rats in HFD + Aloe 20 and HFD + Aloe 200 groups, respectively (p<0.05 for HFD + Aloe 200). DIO rats treated with Aloe 20 and Aloe 200 also exhibited 6.2% and 19.2% reductions in serum concentration of T-Cholesterol compared with HFD-control rats (p<0.05 for Aloe 200, Fig. 3C). Additionally, serum NEFA levels were 15.5% and 18.1% lower than that of HFD-control rats in HFD + Aloe 20 and HFD + Aloe 200 groups, respectively, but these reductions were not significant (Fig. 3B).

**Effects of Aloe vera gel on energy expenditure**

Whole-body oxygen consumption was measured using indirect calorimetry. The DIO rats had lower oxygen consumption (expressed per gram of body weight) than the rats fed with StdD (Fig. 4A). Administration of Aloe vera gel elevated the rate of O2 consumption per gram body weight in DIO rats. RQ, the ratio of carbon produced to oxygen consumed, was extremely low in the HFD-fed animals, reflecting the difference of fat content in the diet, as expected (Fig. 4B). However, administration of Aloe vera gel did not affect RQ in DIO rats.

**DISCUSSION**

Obesity is an abnormal condition of excessive body fat accumulation induced by excess calorie intake. It is recognized that high-fat-containing diets contribute to obesity in both humans and animal models (20–22). Diet-induced obesity (DIO) models have been well established, and widely used to find effective functional foods for anti-obesity. In recent studies, many observations have been reported concerning the ingestion of certain functional foods or their components decreasing the accumulation of body fat and inhibiting the elevation of body weight (23–27).

In the present study, we investigated the effects of Aloe vera gel on a DIO model established by 4 wk of HFD feeding. After the Aloe vera gel treatment period, the body weight in the HFD + Aloe 200 group was 44.5 g lower than that of the HFD-control, and the food intake was reduced by 3.1 g, but these effects were not significant (Fig. 1, Table 1). We speculated that the suppression of body weight gain by Aloe vera gel treatment might bring about a decrease in food intake as a result. Moreover, the ingestion of Aloe vera gel significantly reduced the adipose tissue masses both viscerally and subcutaneously (Fig. 2). When comparing the reduction of specific fat deposits in Aloe vera gel-treated DIO rats, the effect on body weight gain was not clear. However, the reduced body weights observed in Aloe vera gel-treated groups were partially due to a decrease in fat-pad tissues. When the three intra-abdominal fat deposits were compared, the weights of mesenteric and retroperitoneal fat in the HFD + Aloe 200 group were 30.1 and 20.5% lower than those of HFD-control rats, respectively (p<0.05 for mesenteric fat), but the weights of epididymal fat were not changed. It is interesting that Aloe vera gel affects adipose deposits differently. Differences in the site and function of adipose tissues were analyzed by several groups (28, 29). Digirolamo et al. characterized regional changes in adipose tissue growth, and showed a unique capacity of mesenteric adipose deposits for cellular hypertrophy with minimum hyperplasia (28). Caesar et al. found that epididymal fat exhibits low de novo lipogenesis and its expression of androgen receptor (AR) is higher than in mesenteric fat (29). From these observations, it is surmised that the differences in responses to the administration of Aloe vera gel might depend on both regional and characteristic differences in the adipose tissues. Additionally, we speculated that the mesenteric tissues are more influenced than the other deposits by ingredients of Aloe vera gel absorbed intestinally, because of their location.

In DIO model animals, blood TG elevation is commonly observed as an adverse consequence of obesity (30). In our previous study, we already showed that the serum and hepatic TG concentrations in DIO mice were reduced by the treatment of Aloe-sterols (18). Kim et al. also reported that the administration of processed Aloe vera gel (PAG) lowered TG levels in the liver and plasma of diet-induced non-insulin-dependent diabetes mellitus (NIDDM) model mice (31). Consistent with these observations, we confirmed that the administration of Aloe vera gel normalized serum TG levels in DIO rats (Fig. 3). In addition, since the reduction of liver weights was observed in Aloe vera gel-treated DIO rats (Table 2), it is supposed that the hepatic TG contents might be decreased by the administration of Aloe vera gel. Thus, it is suggested that Aloe vera gel exerts anti-obesity and hypolipidemic effects in DIO models.

Moreover, we measured oxygen consumption to address the effect of Aloe vera gel treatment on energy expenditure. We found that O2 consumption per gram body weight of DIO rats was elevated by the Aloe vera gel treatment (Fig. 4). There are some reports that have demonstrated that increase of oxygen consumption and up-regulation of hepatic lipid oxidation genes are observed concomitantly in models of DIO resistance (32, 33). In our previous study, we also demonstrated that the expressions of PPARα (peroxisome proliferators-activated receptor α) and its target enzymes of fatty acid oxidation, such as ACOX (acyl-CoA oxidase) and CPT1 (carnitine palmitoyltransferase-1), were elevated in the livers of Aloe-sterol-treated DIO mice (18). From these results, it is suggested that Aloe vera gel containing Aloe-sterol may act on the liver to stimulate energy expenditure as fatty acid oxidation and contribute to the prevention and improvement of obesity and its related disorders induced by HFD. In addition, it is also supposed that Aloe vera gel may partially interfere with...
intestinal fat absorption for the prevention of hyperlipidemia, although we did not assess the effect on the fecal excretion of lipids.

_Aloe vera_ has a long history as a medicinal plant and has provided a range of the health benefits. Many of the medical effects, such as immune-stimulation, promotion of wound healing, and antibiotic protection, as well as anti-inflammatory, hypoglycemic, and anti-oxidant effects of _Aloe_ leaves have been investigated (8). In particular, the anti-diabetic effects of _Aloe_ gel have been well studied both in humans (11, 12) and streptozotocin-induced animal diabetes models (13, 14, 34).

In the present study, we showed a clear fat pad-reducing effect using a suspension of dried powdered _Aloe vera_ gel at a concentration of 200 mg/kg. On the other hand, Jain et al. reported that homogenized fresh _Aloe vera_ gel at 200 mg/kg exhibited significant anti-diabetic and cardioprotective activities (15). Since raw _Aloe vera_ gel consists of approximately 99.5% water (6), only 0.5–1% of dried _Aloe vera_ gel powder was obtained from raw leaf gel. Therefore, it is assumed that the dosage of _Aloe vera_ gel is 100- to 200-fold different between these two studies. It seems that a large amount of _Aloe vera_ gel is required for a reduction of accumulated body fat; however, we believe the dosage of _Aloe vera_ gel used in our present study would be adequate because we observed that the treatment of _Aloe_ sterols at 25 μg/kg induced an anti-diabetic effect in our previous reports (16, 17). In addition, we confirmed that whole _Aloe_ sterol comprises approximately 0.01% of the _Aloe vera_ gel powder. Consequently, it is calculated that 200 mg of dried _Aloe vera_ gel powder contains 20 μg of total _Aloe_ sterols.

From the inner gel of _Aloe vera_, over 75 active ingredients have been identified and many of health benefits attributed to them, at least in part (35, 36). Although _Aloe vera_ gel contains a large number of bioactive molecules, we suppose that the _Aloe_ sterols are the main active ingredients with an anti-obesity effect in _Aloe vera_ gel. Hence, we have obtained phytosterol-enriched _Aloe vera_ gel extract (AVGE) as a useful ingredient for functional foods. Further studies are required to assess the anti-obesity effect of AVGE.

In conclusion, we demonstrated that the long-term administration of _Aloe vera_ gel induced modest weight loss and reduced body fat accumulation via the stimulation of energy expenditure in a rat model with HFID-induced obesity. These results suggest that _Aloe vera_ gel may prevent and improve obesity caused by a high-fat diet, and its health-promoting effect could be beneficial to reduce the risk of obesity-associated disease, that is, metabolic syndrome.

REFERENCES


Reduction of Body Fat Mass by *Aloe vera* Gel

201


