Ipomoea batatas and Agaricus blazei ameliorate diabetic disorders with therapeutic antioxidant potential in streptozotocin-induced diabetic rats

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Ipomoea batatas, Agaricus blazei and Smallanthus sonchifolius are known to favorably influence diabetes mellitus. To clarify their antidiabetic efficacies and hypoglycemic mechanisms, we treated streptozotocin-induced diabetic rats with daily oral feeding of powdered Ipomoea batatas (5 g kg−1 d−1), Agaricus blazei (1 g kg−1 d−1) or Smallanthus sonchifolius (4 g kg−1 d−1) for 2 months. Treatments with Ipomoea batatas or Agaricus blazei, but not Smallanthus sonchifolius, significantly suppressed the increases of fasting plasma glucose and hemoglobin A1c levels, and restored body weight loss during diabetes. Serum insulin levels after oral glucose administration tests increased along the treatments of Ipomoea batatas or Agaricus blazei. Moreover, Ipomoea batatas and Agaricus blazei reduced superoxide production from leukocytes and vascular homogenates, serum 8-oxo-2′-deoxyguanosine, and vascular nitrotyrosine formation of diabetic rats to comparable levels of normal control animals. Stress- and inflammation-related p38 mitogen-activated protein kinase activity and tumor necrosis factor-α production of diabetic rats were significantly depressed by Ipomoea batatas administration. Histological examination also exhibited improvement of pancreatic β-cells mass after treatments with Ipomoea batatas or Agaricus blazei. These results suggest that hypoglycemic effects of Ipomoea batatas or Agaricus blazei result from their suppression of oxidative stress and proinflammatory cytokine production followed by improvement of pancreatic β-cells mass.

Key Words: hypoglycemic effect, oxidative stress, pancreatic islet β-cells, Ipomoea batatas (Caiapo), Agaricus blazei (Agarics)

Nowadays, diabetes mellitus in the industrialized countries has spread to younger generation and the number of patients is expected to increase to 300 million or more worldwide by the year 2025. Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. Even optimal control of blood glucose can not prevent clinical diabetic complications, so that diabetes therapy has revolved around dietary measures including the use of traditional antihyperglycemic medicines. Moreover, insulin or oral hypoglycemic drugs have serious side effects on overdose medication. Therefore, there is a real need today for further pharmacological study on the effectiveness and side effects caused by the alternative medicines.

In fact, about 800 species of plants and mushrooms have been reported to improve the metabolism of carbohydrates and to be effective against diabetes mellitus.(15–3) However, only a few compounds of antihyperglycemic plants and mushrooms have shown the efficacy on the management of diabetes in randomized trials.(3,4) Their hypoglycemic mechanisms in chronic treatment are not clear and scientific evidences are very poor. In the present study, we selected the three of plants and mushroom based on the previous reports, Ipomoea batatas L. (Convolvulaceae), i.e., Caiapo, Agaricus blazei Murill (Agaricaceae), i.e., Agarics, and Smallanthus sonchifolius Poepp. & Endl. (Compositae), i.e., Yacon, which may improve on the balance of glucose and insulin through a safe and effective program.

Ipomoea batatas is a white-skinned sweet potato originating in Amazonos Brazil, and it has been used in Shikoku region of Japan, as a folk medicine for the treatments of diabetes and other metabolic diseases.(3,4) In concerning diet therapy of Ipomoea batatas, the treatment for 3 months has been reported to lower the plasma glucose and cholesterol levels in patients with type 2 diabetes.(7,8) Oral administration of Ipomoea batatas to diabetic animal models for longer than 6 weeks was shown to prevent and improve the symptoms of diabetes and hypoglycemia in streptozotocin (STZ)-induced diabetic and obese Zucker rats.(9,10)

Further, Ipomoea batatas has abundant phenolic compounds, such as caffeic acid and its derivatives,(11) whose efficacious functions(12,13) are expected to prove a number of health benefits. Agaricus blazei is a medical mushroom that grows in North America and Brazil, and it is widely taken, and described in the world.(13) There are many reports on the immunological beneficial properties such as anti-tumor,(14,15) anti-viral,(16) and disinfectant(17) activities. Moreover, it is recently reported that β-glucan extracts from Agaricus blazei could reduce blood glucose, triglyceride, and cholesterol levels(18) as well as insulin-like action.(19)

Smallanthus sonchifolius roots are a rich source of fructose-oligosacharides and have a long use tradition as food in the Andean region. Expansion to other counties including New Zealand, Japan and Brazil has been stimulated further by presumed medical properties of both roots and leaves.(20) The water-soluble extract of its leaves has been recognized to have hypoglycemic effect,(21,22) antioxidant property,(23) and enhancement of liver metabolism.(24) Natural and nutriceutical products including the above three materials are highly expected on diabetes control.

In the present study, Ipomoea batatas, Agaricus blazei, or Smallanthus sonchifolius were fed with diet for 2 months to STZ-induced type 2 diabetes rats, and their efficacy against diabetes and diabetes-associated pathophysiology changes including pancreatic β-cells, insulin secretion, oxidative stress, and inflammatory cytokine production were investigated.

Materials and Methods

Preparation of experimental materials. Ipomoea batatas (Caiapo) was grown in Kagawa Prefecture (Japan). Whole extract

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was obtained including the peel, because Ipomoea batatas contains more phenolic compounds in the outer part than in the center;\(^{(25)}\) and then lyophilized to prepare a uniform powder.\(^{(26)}\) The powder was purchased from Fuji Sangyo Co., Ltd. (Kagawa, Japan). All ingredients were measured by Japan Food Research Laboratories (Tokyo, Japan) using the standard protocols recommended by the Resources Council, the Science and Technology Agency of Japan. The main composition was as follows: protein, 5.2%; carbohydrate, 78.3%; fiber, 11.0%. *Ipomoea batatas* had also abundant phenolic compounds such as chlorogenic acid, caffeic acid and its derivatives,\(^{(27)}\) and high levels of vitamins including ascorbic acid and tocopherol. It contained many minerals including zinc, copper, manganese and selenium.

*Agaricus blazei* (*Agaricus*) fruit body was cultivated outdoors in Brazil. Fruit bodies were air-dried by ventilator with a blowing temperature low than 60°C to maintain their enzyme activities, powdered, and imported by Toei Pharmaceutical Co., Ltd. (Tokyo, Japan). The main composition was as follows: protein, 38.5%; carbohydrate, 27.7%; fiber, 20.6%; \(\beta\)-glucan, 12.4%. It had also enzymes such as polyphenol oxidase, peroxidase, glucanase, and laccase. It contained many minerals including large amounts of zinc and copper, and certain amounts of manganese and selenium.

Smallanthus sonchifolius (*Yacon*) plants were grown in Brazil. The tuberous roots and stems of *Smallanthus sonchifolius* were peeled, sliced and dried at 40°C in a forced air circulation oven. Partially dried slices were subjected to 60°C for 2 h to stop rapid degradation of fructooligosaccharides,\(^{(28)}\) milled, and imported by Toei Pharmaceutical Co., Ltd. The main composition was as follows: protein, 9.6%; carbohydrate, 66.0%; \(\beta\)-glucan, 11.6%. The major proportion of carbohydrates was in the form of inulin-type oligofructans and \(\beta\)-fructooligosaccharides.\(^{(29)}\) The tubers had chlorogenic acid and caffeic acid derivatives.\(^{(30)}\) It also contained ascorbic acid, and many minerals including zinc, copper, manganese and selenium.

All diets for animals were prepared by mixing powdered test materials and powdered standard diet (SP; Funabashi Farm, Funabashi, Japan). The doses of *Ipomoea batatas*, *Agaricus blazei*, or *Smallanthus sonchifolius* were kept at 5 g/kg of body weight/day, 1 g/kg of body weight/day, or 4 g/kg of body weight/day, respectively. This is a dose likely to be in the upper limit of what can be tolerated in long-term experiments in human as indicated by the manufacturers.

**Animals.** Male Wistar rats weighing 130 g to 150 g (6 weeks of age, Japan SLC, Inc., Shizuoka, Japan) were housed in an air-conditioned room at 22 ± 2°C with 60 ± 5% humidity, under 12 h light-dark cycle in the center for animal experiments of Kinki University School of Medicine. Rats were given laboratory diets as mentioned above and water *ad libitum*. Rats were treated according to the ethical guideline of Kinki University School of Medicine Animal Committee.

**Experimental procedure and sample collection.** After initial determination of fasting blood glucose levels at 7 weeks of age, rats were intravenously injected the streptozotocin fluid (STZ, Sigma Chemical Co., St. Louis, MO) at the dose of 45 mg/kg (freshly dissolved in 3 mM citrate buffer pH 4.5). Blood glucose levels were monitored on the fifth day after STZ injection and thereafter at a fasting period of 18 h prior to the monitoring once a week. Animals showing above 250 mg/dl of blood glucose levels on the fifth day after STZ injection were selected as diabetic rats for this study and randomly divided into 4 groups: (i) normal diet (n = 5), (ii) *Ipomoea batatas* diet (n = 5), (iii) *Agaricus blazei* diet (n = 5), (iv) *Smallanthus sonchifolius* diet (n = 5). The treatments with *Ipomoea batatas*, *Agaricus blazei* or *Smallanthus sonchifolius* were performed for 8 weeks during 8 to 15 weeks of age. For normal reference, non-diabetes control (n = 5) was also examined.

Body weight of normal and diabetic rats was weekly recorded in fasting state. Food and water intake, and urine output for 24 h were measured every other week (1, 3, 5, and 7th week after treatments) individually in metabolic cages. Blood samples were collected from the tail vein. Urine and serum samples were stored at −80°C until the assay. Animals were sacrificed under pentobarbital anesthesia in the eighth week after treatments, and the pancreas, kidney and aorta were collected for the pathophysiological examinations of protein.

**Plasma glucose and HbA1c.** Plasma glucose values were obtained by the electric tip using glucose oxidase method\(^{(31)}\) (MediSense Xtra; Abbott Laboratories Inc., Tokyo, Japan). Levels of glycosylated hemoglobin A1c (HbA1c) and total hemoglobin\(^{(32)}\) after 7 weeks of treatments were determined according to manufacturer’s instruction (Liquitech HBAlcII; Roche Diagnostic Systems, Branchburg, MO).

**Serum insulin.** Serum insulin levels 20 min after oral administration of 20% glucose solution were determined by radioimmunoassay\(^{(33)}\) using Rat Insulin radioimmunoassay (RIA) kit (Linco Research Inc., St. Charles, MO) 1, 3, 5 and 7 weeks after treatments. Fasting insulin levels were also measured in rats fasted overnight in the eighth week after treatments using Ultrasensitive Rat Insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden).

**Superoxide production by peripheral leukocytes.** Superoxide production by polymorphonuclear leukocytes (PMNs) and monocytes (MNCs) was measured using a gated flow cytometry according to the methods described by Perticarari et al.\(^{(34)}\) with some modifications. Seven weeks after treatments fresh blood was collected and erythrocytes were removed by Tris-buffered ammonium chloride lysis. White blood cells were preincubated for 15 min in a shaking water bath in 37°C with 500 ng/ml hydroethidium (HE) (Invitrogen Life Technologies, Carlsbad, CA) in Hank’s Balanced Salt Solution containing 1% bovine serum albumin (Sigma Chemical Co.), and then the reaction was immediately stopped on ice. HE, a nonfluorescent compound which can diffuse through cell membrane, is rapidly oxidized to ethidium bromide by oxidative products, giving red fluorescence emission. Superoxide levels produced by PMNs and MNCs were evaluated by mean fluorescence intensity. Superoxide levels in stimulated leukocytes in the presence of 1 \mu M phorbol myristate acetate (PMA; Sigma-Aldrich Co.) were also measured.

**Lucigenin-enhanced chemiluminescence detection of vascular superoxide.** Vascular superoxide production was measured using lucigenin (bis-N-methyl acridinium; Sigma-Aldrich Co.)-enhanced chemiluminescence as described by Guzik et al.\(^{(35)}\) Chemiluminescence in the vascular homogenates 8 weeks after treatments was measured in Krebs-HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) buffer containing lucigenin (100 \mu M) using luminometer analyzer (PICO-LITE 6100; Packard Instrument Co. Inc., Downers Grove, IL). Specificity for superoxide was determined by coinoculation with tiron (20 mM) (4,5-dihydroxy-1,3-benzene disulfonic acid; Sigma-Aldrich Co.). Superoxide production was expressed as chemiluminescence counts/10 min/30 mg vessel wet weight.

**Serum 8-oxo-2′-deoxyguanosine (8-OHdG).** Serum samples of 8 weeks after treatments of the experimental diets were filtrated to remove high molecular weight products above 10,000 MW (Ultrafree-MC; Millipore Co., Bedford, MA). Thereafter, 8-OHdG levels of the samples were determined\(^{(36)}\) by high sensitive commercial ELISA kit (Japan Institute for the Control of Aging, Nikken SEIL Co., Shizuoka, Japan).

**Urinary Tumor necrosis factor-α (TNF-α).** Urine samples of 5 and 7 weeks after treatments of the experimental diets were centrifuged at 5,000 \times g for 10 min. TNF-α level in the supernatants was measured\(^{(37)}\) by BD Opt EIA Rat TNF ELISA kit II (BD biosciences, San Jose, CA).

**Western blot analysis.** Protein from aortas were extracted in a lysis buffer containing 0.1% sodium dodecylsulfate (SDS),
Yacon groups during the experimental period. Differences in food intakes among DC, Caiapo, Agaricus and any groups (data not shown). There were also no significant thereafter there were no significant differences observed among diabetic groups for 3 weeks after the start of treatments, and significant restoration of the body weight.

Smallanthus sonchifolius

Body weight.

Fasting blood glucose levels of NC ranged from 82.5 ± 9.4 mg/dl to 111.2 ± 2.6 mg/dl during the experiment. On the other hand, blood glucose levels of DC were increased after injection of STZ, and 5 weeks later those reached to the peak level (474.2 ± 34.1 mg/dl). In comparison with DC, Caiapo and Agaricus groups showed significant suppression on blood glucose levels after 3 weeks of treatments (p<0.05, Caiapo or Agarics group vs DC). In contrast, Yacon showed no significant hypoglycemic effect during the experimental period (Fig. 2).

There was a significant increase in the level of glycosylated HbA1c in DC after 7 weeks of STZ injection compared with that of NC (p<0.01). Of note, the value of Caiapo group was suppressed to 4.1 ± 0.2%, which was approximately the same level as NC (p<0.01 vs DC, 7.6 ± 0.8%). Suppressive tendency was also observed in Agaricus group (5.8 ± 0.7%). In contrast, HbA1c level of Yacon group did not differ from that of DC (Fig. 3). Furthermore, urinary ketone levels were increased in all of the diabetic groups compared with NC after 5 weeks of STZ injection. The levels were suppressed in Caiapo and Agaricus groups compared with DC. On the other hand, Yacon had no effect on urinary ketone (data not shown).
Serum insulin levels after glucose loading. Changes of serum insulin levels 20 min after 400 mg/rat glucose loading were shown in Fig. 4. In the first week of experiment, mean serum insulin levels in all of the diabetic groups were remarkably decreased to 0.74 ng/ml (p<0.01 vs NC, 2.3 ± 0.4 ng/ml). After 5 weeks of treatments with the experimental diets, the increases in insulin levels were observed in Caiapo and Agaricus groups after glucose loading. The levels of Caiapo groups was significantly higher compared with DC (p<0.05). In the seventh week after treatment, the mean value of Caiapo group was 2.7 ng/ml, and thus recovered to the level comparable to that of NC, although the error range was great (p<0.05 vs DC). By contrast, the levels of serum insulin in Yacon group did not differ from those of DC during the experiment. On the other hand, fasting insulin levels remained unchanged in all the diabetic groups.

Oxidative stress. One major mechanism underlying STZ toxicity is cytokine-mediated β-cell destruction in which oxidative stress plays a key role. First, we examined the changes of superoxide production from leukocytes in normal or diabetic rats with or without the treatments of Caiapo, Agaricus, or Yacon for 8 weeks. Mean fluorescence intensity, which is equivalent to the ability of superoxide production by a single monocyte or neutrophil, under phorbol mirystate acetate stimulation (1 μM) was measured in the serum from normal or diabetic rats. The levels were determined 20 min after oral administration of 20% glucose solution by radioimmunoassay. Treatment with Caiapo for 2 months restored to serum insulin level comparable to that of normal control. Normal control (filled square), untreated diabetic control (filled triangle), Agaricus-treated diabetic (open circle), and Yacon-treated diabetic (open triangle) rats. Values are mean ± SEM (n = 5). *p<0.01 vs normal control, **p<0.05 vs untreated diabetic control.

Fig. 3. Levels of HbA1c in STZ-induced diabetic rats treated with Ipomoea batatas (Caiapo), Agaricus blazei (Agarics) or Smallanthus sonchifolius (Yacon) in the seventh week after treatments. HbA1c values were reduced with treatment with Caiapo. Values are mean ± SEM (n = 5). *p<0.01 vs normal control, **p<0.01 vs untreated diabetic control. NC; normal control, D + None; untreated diabetic control, D + Caiapo; Ipomoea batatas-treated diabetic group, D + Agarics; Agaricus blazei-treated diabetic group, D + Yacon; Smallanthus sonchifolius-treated diabetic group.

Fig. 4. Serum insulin levels after oral glucose administration in Ipomoea batatas-treated diabetic (Caiapo), Agaricus blazei-treated diabetic (Agarics) or Smallanthus sonchifolius-treated diabetic (Yacon) rats. The levels were determined 20 min after oral administration of 20% glucose solution by radioimmunoassay. Treatment with Caiapo for 2 months restored to serum insulin level comparable to that of normal control. Normal control (filled diamond), untreated diabetic control (filled square), Caiapo-treated diabetic (filled triangle), Agaricus-treated diabetic (open circle), and Yacon-treated diabetic (open triangle) rats. Values are mean ± SEM (n = 5). *p<0.01 vs normal control, **p<0.05 vs untreated diabetic control.

Fig. 5b). Caiapo for 8 weeks significantly attenuated the formation (p<0.05, Fig. 5b).

Fig. 6. Urinary TNF-α level 5 and 7 weeks after treatments was illustrated in Fig. 6. In the fifth week of treatment, urinary TNF-α level of the diabetic rats was increased by 6 to 9 times as high as that of NC (p<0.01, NC 23.6 ± 6.3 pg/ml vs DC 180.4 ± 11.2 pg/ml). The levels of Caiapo (141.1 ± 10.6 pg/ml) and Agarics (150.9 ± 6.2 pg/ml) groups were significantly less than that of DC (p<0.05 vs DC). In contrast, Yacon group remained unchanged. In the seventh week, TNF-α levels showed similar tendencies with those of the fifth week, although significant differences between the groups were not observed. We also measured the levels of serum TNF-α in the experimental groups. The values were low (range of the mean from 9 pg/ml to 13 pg/ml) and were not significantly different between the groups.

Mean urinary albumin was increased to 4.02 mg/day urine albumin excretion (UAE) in DC group 5 weeks after the experiment. Caiapo, Agaricus or Yacon reduced UAE to the level comparable to that of NC group (1.5 mg/day mean UAE) (data not shown).

After 1 week of the experiment, water intakes in all of the diabetic rat groups were increased by 3-fold as much as that of NC, and their urine excretions were also increased by 5-fold, with no difference between DC and the other treated diabetic groups. In addition, the ratio of kidney/body weight 8 weeks after treatments became approximately twice in the diabetic rat groups.
compared with that of the NC, with no difference between DC and the other treated diabetic groups (data not shown).

**Activation of p38 mitogen-activated protein kinase.**

Data of the superoxide production and TNF-α levels were measured in the urine of 5 and 7 weeks after the treatments using enzyme-linked immunosorbent assay. Urinary TNF-α excretion was markedly increased in untreated diabetic rats. Caiapo and Agaricus significantly reduced urinary TNF-α excretion after 5 weeks with treatments. Values are mean ± SEM (n = 5). *p<0.01 vs normal control, *p<0.05 vs untreated diabetic control. NC; normal control, D + None; untreated diabetic control, D + Caiapo; Ipomoea batatas-treated diabetic group, D + Agarics; Agaricus blazei-treated diabetic group, D + Yacon; Smallanthus sonchifolius-treated diabetic group.

**Discussion**

*Ipomoea batatas* L. (Caiapo), *Agaricus blazei* Murill (Agaricus), and *Smallanthus sonchifolius* Poepp. & Endl. (Yacon) are known to favorably influence diabetes mellitus. However, their antidiabetic efficacy and hypoglycemic mechanisms are not fully determined. In the present study, we have shown that the treatments of *Ipomoea batatas* and *Agaricus blazei* suppress the increase in oxidative stress and TNF-α production in STZ-induced diabetes, and are useful in pancreatic β-cells recovery.

There has been much recent attention given to the relationship between diabetes and oxidative stress. Excessive superoxide production is likely to injury pancreatic β-cells and to weaken the insulin production. In this study, superoxide production by peripheral blood monocytes and neutrophils was increased in diabetic rats, accompanied by an increase in the levels of serum 8-OHdG, which displays cellular DNA damage as described in the text. We also demonstrated that the levels of plasma glucose and HbA1c were correlated with increased oxidative stress in diabetic rats. These findings are coincident with the reports that superoxide production and 8-OHdG levels from peripheral blood...
monocytes were significantly higher in diabetic patients than in healthy people, and that there was a correlation between the rise of HbA1c and oxidative stress levels from peripheral blood monocytes in diabetic patients. Here, it is of note that long-term treatments with *Ipomoea batatas* or *Agaricus blazei* significantly suppressed the increase in HbA1c and oxidative stress in diabetes animals (Figs. 1 and 3).

Furthermore, Sheetz and King and Aslan et al. have reported that free radicals induced oxidative stress-related gene expressions and peroxidization of plasma membrane, and that contributed to the development of various diabetic complications. Consistently, the present study showed that oxidative stress levels in the aortas of diabetic rats were markedly increased, as shown in the results in superoxide production (Fig. 5a) and nitrotyrosine
formulation (Fig. 5b). Nitrotyrosine is a footprint of increased peroxynitrite presence, which is the reactive product of superoxide and nitric oxide, and is a highly reactive radical species. Lakey et al.\(^\text{[59]}\) have reported that peroxynitrite is a mediator of cytokine-induced destruction of human pancreatic islet β-cells. Of profound importance, treatments with Ipomoea batatas or Agaricus blazei significantly reduced the production of reactive oxygen species in vascular cells as well as peripheral blood cells.

Recent study shows the evidence for evaluation of nitrotyrosine and TNF-α as potential biomarkers of the presence, severity and progression of diabetes and its complication.\(^\text{[53]}\) TNF-α, a pro-inflammatory cytokine, is mainly secreted from monocytes and macrophages, and also produced in various other tissues. It has reported that TNF-α affects not only peripheral insulin resistance,\(^\text{[52]}\) but also directly pancreatic β-cells, followed by their apoptosis and reduction of insulin secretion.\(^\text{[53]}\) It has also demonstrated that high glucose participates in the production of inflammatory cytokines including TNF-α through the activation of nuclear transcription factor.\(^\text{[54]}\)

Here we show that TNF-α production remarkably increased in STZ-induced diabetic rats and that treatments of Ipomoea batatas or Agaricus blazei had significant suppressive effect on urinary TNF-α level (Fig. 6). Additionally, Ipomoea batatas deprived p38 MAP kinase expression and activation (Fig. 7). P38 MAP kinase is potently and preferentially activated by a variety of stress including oxidative stress and inflammatory cytokines and receptor systems of the TNF family.\(^\text{[45]}\) Excretion of urinary albumin was also significantly increased in diabetic rats and suppressed after 5 weeks of treatments with Ipomoea batatas, Agaricus blazei, and Smallanthus sonchifolius. It supports our result that increase in urinary TNF-α level is associated with the onset of diabetic nephropathy.\(^\text{[48]}\)

Ipomoea batatas-treated and Agaricus blazei-treated rats exhibited the recovery tendency on serum insulin levels along the treatments (Fig. 4). In the histochemical examination of pancreatic islets (Table 1, Fig. 8), a recovery tendency of islet β-cells mass was also observed in Ipomoea batatas or Agaricus blazei groups. These results suggest that there is a possibility that Ipomoea batatas or Agaricus blazei has a protective effect on islet cell destruction and increases the secretion of insulin. On the other hand, Kusano and Abe\(^\text{[10]}\) have reported that Ipomoea batatas extract directly secretes insulin and improves insulin resistance. Gray and Flatt\(^\text{[19]}\) have found the insulin-like activity and the insulin-releasing effect of Agaricus blazei, although the nature or the mechanisms of action was not determined.

Taken together, hypoglycemic effects of Ipomoea batatas or Agaricus blazei are likely to be their antioxidant and anti-inflammatory action. The leaves and roots of Ipomoea batatas used in this study have abundant phenolic compounds, such as chlorogenic acid, caffeic acid, and its derivatives,\(^\text{[11]}\) which exhibit high radical-scavenging activity, antimutagenic, and anti-diabetic effects.\(^\text{[15,16]}\) Ipomoea batatas is rich in ascorbic acid, a well-known antioxidant molecule. The plant also contains large amounts of dietary fibers, which may interfere with glucose absorption and further reduce blood glucose levels.

On the other hand, the fruit body of Agaricus blazei has highly branched 1,3-glucan as the major carbohydrate component.\(^\text{[55]}\) β-glucan is a well-known biological response modifier which is widely distributed in nature and used as an alternative medicine. Although the previous reports concerning the oxidative/anti-oxidative activity of β-glucan are controversial, β-glucan treatment was found to be effective against oxidative injury through the inhibition of TNF-α response.\(^\text{[58–60]}\) In addition, Agaricus blazei contains polyphenol oxidase, an antioxidant enzyme.\(^\text{[28,29]}\) Copper, zinc, and selenium contained richly in Ipomoea batatas and Agaricus blazei may be beneficial for the protection against radical generation, because the minerals have radical-scavenging activities and are contained in antioxidant enzymes. It is important that neither significant adverse effects nor biochemical changes of blood were observed in our other experiment using Ipomoea batatas, Agaricus blazei, or Smallanthus sonchifolius.

In opposition to previous reports,\(^\text{[21,22]}\) Smallanthus sonchifolius did not improve in glucose balance, oxidative stress or inflammatory response. We used primarily dried tuber and stem powder of Smallanthus sonchifolius in this study, and there may be a possibility of discrepancy between our results and the results of other reports which utilized the decoction from the leaves.

Collectively, we provide that cellular oxidative is a critical step in STZ-mediated islet β-cells injury, and that Ipomoea batatas and Agaricus blazei protect the organs from oxidative damage by those immunomodulatory (inhibition of TNF-α formation and its signaling pathway) and antioxidant properties. Excessive NO production is recently thought to be involved in the pathogenesis of metabolic disorders such as atherosclerosis and obesity-linked type 2 diabetes.\(^\text{[61,62]}\) Proinflammatory cytokines including TNF-α increase nitric oxide (NO) production through the expression of inducible NO synthase in adipocytes and in skeletal muscle of obese human subjects and several diabetic animal models.\(^\text{[62,63]}\) In the present study using STZ-induced diabetic rats, rise of plasma or urine NOx levels was not detected. However, we do not exclude the possible mechanism, because nitrotyrosine formation was markedly increased in vascular cells of untreated diabetic rats (Fig. 5b).

In conclusion, treatments with Ipomoea batatas or Agaricus blazei suppressed the increases of blood glucose and HbA1c levels in streptozotocin-induced type 2 diabetes model rats, and restored insulin release and their body weight loss. The results suggest that the antidiabetic effects result from the suppression of oxidative stress and proinflammatory cytokine, TNF-α, and improvement in β-cells mass. Further precise mechanism of suppression on oxidative stress and/or TNF-α production by the components of Ipomoea batatas or Agaricus blazei remains to be elucidated.

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**Table 1. Histochemical analysis of pancreatic islets in diabetic rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Islet number</th>
<th>β-cells area (mm², ×1/100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>3.77 ± 0.09</td>
<td>3.58 ± 0.23</td>
</tr>
<tr>
<td>D + None</td>
<td>1.06 ± 0.12**</td>
<td>0.56 ± 0.17**</td>
</tr>
<tr>
<td>D + Caiapo</td>
<td>1.46 ± 0.12*</td>
<td>1.03 ± 0.30</td>
</tr>
<tr>
<td>D + Agaric</td>
<td>1.52 ± 0.11*</td>
<td>1.25 ± 0.35</td>
</tr>
<tr>
<td>D + Yacon</td>
<td>0.96 ± 0.08</td>
<td>0.68 ± 0.21</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 5). *p<0.01 vs NC, **p<0.05 vs DC.

Twelve fields were examined in each animal. Islet number is count pancreatic islets per field. Positive staining area by aldehyde fuchsin for an islet is calculated as β-cells area.

NC, normal control; D, None; untreated diabetic control; D + Caiapo; Ipomoea batatas-treated diabetic group; D + Agaric; Agaricus blazei-treated diabetic group; D + Yacon; Smallanthus sonchifolius-treated diabetic group.

Twelve fields were examined in each animal. Islet number is count pancreatic islets per field. Positive staining area by aldehyde fuchsin for an islet is calculated as β-cells area.
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**Abbreviations**

ANOVA analysis of variance
dc untreated diabetic control
desa enzyme-linked immunosorbent assay
HbA1c hemoglobin A1c
He hydroethidium
hepes N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
map kinase mitogen-activated protein kinase
MNCs monocytes
nadph nicotinamide adenine dinucleotide phosphate
nc normal control
no nitric oxide
8-OhdG 8-oxo-2-deoxyguanosine
pma phorbol myristate acetate
PMN{s} polymorphonuclear leukocytes
ria radioimmunoassay
sds sodium dodecylsulfate
sem standard error of the mean
stz streptozotocin
TNF-α tumor necrosis factor-α
UAE urine albumin excretion

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