Anti-diabetic Activity Present in the Fruit Body of *Grifola frondosa* (Maitake). I

Keiko KUBO, a,1) Hisao AOYI, b and Hiroaki NANBA* c

Yukiguni Maitake Co., Ltd., a, Mukamachi, Minamiaomori, Niigata 949-66, Japan, Aoki Clinic,b Ninomiya, Chiyō, Kobe, 651, Japan, and Department of Microbial Chemistry, Kobe Pharmaceutical University,c Motoyama, Higashinada, Kobe 658, Japan. Received March 7, 1994; accepted April 28, 1994

The fruit body of *Grifola frondosa* (maitake), Basidiomycetes was confirmed to contain substances with anti-diabetic activity. When 1 g/d of powdered fruit body of maitake was given orally to a genetically diabetic mouse (KK-A' ), blood glucose reduction was observed, in contrast to the control group in which the blood glucose increased with ageing. Moreover, levels of insulin and triglyceride in plasma demonstrated a change similar to blood glucose with feeding of maitake. Ether–ethanol-soluble (ES) and hot water-soluble (WS) fractions were prepared from the fruit body and their hypoglycemic activity was examined. Blood glucose-lowering activity was found when ES-fraction or WS-50% ethanol float (X) fraction was administered orally, but other WS-fractions were inactive. These results suggest that the anti-diabetic activity was present not only in the ES-fraction consisting of lipid but also in the X-fraction of peptidoglycan (sugar : protein = 65 : 35).

Keywords *Grifola frondosa* (maitake); anti-diabetic activity; KK-A' mouse; NIDDM; blood glucose

It has been reported that various biologically active materials exist in the fruit bodies of mushroom (Basidiomycetes). 2) Crestin extracted from kawaratake (Cortious versicolor),3) and Lentinan from shiitake (Lentinus edodes)4–7) are high molecular compounds which have shown antitumor activity and have already been used clinically. The authors have reported previously that a glucan obtained from maitake (*Grifola frondosa*), possesses antitumor activity by both intraperitoneal and oral administration,8–10) and that diethyl ether-soluble fraction from maitake possesses blood pressure lowering activity.11) It was recently reported that mannentake (*Ganoderma lucidum*), one of the Basidiomycetes, has blood glucose-lowering activity.12,13) The authors’ examination of whether a material showing the same blood glucose-lowering activity is present in maitake showed that maitake demonstrated even stronger activity than mannentake.

**MATERIALS AND METHODS**

**Preparation of Solid Feed with Powdered Maitake**

Fruit bodies of maitake were dehydrated at 65 °C for 4 h and pulverized. (φ200 μ). This maitake powder was mixed with commercial food, CRF-1 (Charles River Co.) in a ratio of 1:4 and distilled water was added (800 ml/kg). After thorough kneading, 3 × 3 cm squares were cut which

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![Diagram](image-url)

**Fig. 1. Fractionation Procedure of *Grifola frondosa* (Maitake)**

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were dehydrated at 80°C for 20 h and 20% maitake solid feed (20% M-feed) was prepared.

**Preparation of Extract Fraction** Various fractions were obtained in the procedure as illustrated in Fig. 1. Diethylether–ethylalcohol soluble-fraction (ES-fraction) was obtained after powdered maitake (300 g) was extracted under reflux with 1500 ml of diethylether and ethylalcohol (1:1) mixture at 70°C for 6 h. The residue was extracted with 3000 ml of distilled water to obtain hot water soluble material was added with ethylalcohol to make a final concentration at 50%, then allowed to stand at 4°C for 12 h to collect the float (X-fraction). After elimination of X-fraction, ethanol was again added to make a final concentration at 80% in order to obtain a high molecular precipitate (YP-fraction) and a low molecular supernatant (YS-fraction), respectively.

**Preparation of Solid Feed with Maitake Fraction** Each fraction obtained from 300 g dried maitake powder as above was concentrated and added, with 1500 g of powdered feed and 20% solid feed, which included various maitake extracts obtained by the same method. Only ES-fraction was prepared by dissolving it with the diethylether–ethylalcohol mixture, adding it to the solid feed and dried at 80°C.

**Animals** Seven week old female spontaneously diabetic mice (KK-A'Y) purchased from Clea Japan Inc. were raised on laboratory chow (CRF-1, Japan Charles River Co.) and water ad libitum in a temperature-controlled room, 24 ± 1°C and 55% humidity under specific pathogen-free conditions for one week in our laboratory before being used in this experiments.

**Chemical Analysis** For blood sampling, orbital sinus was cut by a heparinized hematocrit tube, and blood was collected in this tube. The capillary tubes were centrifuged at 7000 rpm for 4 min and the separated plasma was frozen until assayed. Glucose B-test Wako for plasma glucose, Triglyceride E-test Wako for triglyceride and Insulin-EIA test kits for insulin were obtained from commercial products.

**Statistical Examination** Student's t-test was used to examine the significance of differences in the measured values of each group.

**RESULTS**

Whether or not fruit bodies of maitake possess blood glucose-lowering activity was examined. Female KK-A'Y mice (8 weeks old) were fed on 20% M-feed and their blood glucose levels were measured periodically. The glucose level of the M-feed group was 200 mg/dl after 8 week period showing little change from the start of the experiment, while the level of mice in the control group on normal feed (N-feed) rose to 400 mg/dl (Fig. 2a). This suggests that maitake inhibits a rise in the blood glucose level.

The insulin level in plasma increased to 1200 μU/ml in a straight manner in mice of the control group after the 7th week, while mice in the M-feed group showed 1/3—1/2

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**Fig. 2. Profiles of Biochemical Change on Diabetic KK-A'Y**

Significance of differences (t-test): a) p < 0.05, b) p < 0.01. ---○---, control; ---●---. maitake.
Fig. 3. Effects of Maitake on Anti-diabetic Activities for KK-A' Mice
Significance of differences (t-test): a) p < 0.05, b) p < 0.01.

Fig. 4. Effects of Maikake-Extracts on Glucose-Level in Blood of Diabetic KK-A' Mice
(a) control; (b) YS-fraction; (c) ES-fraction; (d) YP-fraction (e) X-fraction. Significance of differences (t-test): a) p < 0.05, b) p < 0.01.
level of the control group, though there were moderate increases was observed (Fig. 2b).

Also, the triglyceride level in plasma of the control group increased to 780 mg/dl in the 8th week, double that at since the start of the experiment in the same manner as the blood glucose level, while that of the M-feed group remained almost unchanged at 410 mg/dl (Fig. 2c).

The weight of KK-A' mouse which has an obesity gene increases as it grows. However, weights of the M-feed group were about 10 g lighter than those of the control group (Fig. 2d).

Significance of difference in statistics exists between all these measurements. Whether the difference in weight between the control and M-feed groups affects the biochemical values obtained in the experiments was investigated. Feed was altered and 20% M-feed was fed to the control group beginning the 5th week, and N-feed was given to the M-feed group. The results are shown in Fig. 3.

Blood glucose of mice in the control group which had risen to 400 mg/dl decreased to 230 mg/dl the week after the administration of M-feed began. This was close to the start-up level and when little weight change had yet been seen. After 2 weeks, it decreased further to 155 mg/dl which was below the start-up level (Fig. 3a, d). The levels of insulin and triglyceride demonstrated similar changes as when diet was altered from N-feed to M-feed; Insulin level decreased to 20 from 790 μU/ml and triglyceride dropped from 560 to 125 mg/dl in a single week (Fig. 3b, c). These reductions were significant. Blood glucose of mice in the M-feed group which had maintained a level below the start-up level increased quickly when feed was changed from M to N-feed; after one week, the value had significantly changed to 365 from 180 mg/dl as shown in Fig. 3a. The levels of insulin increased to 990 from 120 μU/ml and that of triglyceride demonstrated to 620 from 230 mg/dl in Fig. 3b, c. Thus, after 1 week of the change to N-feed, there was first a quick drop in insulin level followed by a decrease in glucose level, before a slight weight increase was seen. Otherwise, no body-weight increase was found even if the M-feed was changed to N-feed, although during this period levels of insulin, glucose and triglyceride were increased very rapidly. This indicates that the changes in values occurred prior to the changes in body weight.

To examine which component of maitake was responsible for these activities, materials in the ES, X, YS and YP-fractions were mixed with N-feed at an inclusion of 20% of the total feed. As shown in Fig. 4, the blood glucose had decreased only in mice in the ES-feed and X-feed groups after 4 weeks. These glucose levels remained low during the period when these feeds were offered.

The blood glucose-lowering activities by the ES and X-fractions were also examined with the results shown in Fig. 5. Though the glucose levels rose moderately in both the ES and X-feed groups, they were only about 1/2 that of the control group at the 4th week (Fig. 5a). Insulin levels of these groups were 1/3—1/6 (Fig. 5b) and those of triglyceride were about 1/4 (Fig. 5c) those of the control group. Since there was little difference in weight among these three groups an agent to demonstrating anti-diabetic activity is believed to be present in the components of the ES and X-fractions.

DISCUSSION

In studies on the biological activities of mushrooms belonging to Basidiomycetes have been conducted. The authors earlier reported on Grifola frondosa.8 In 1986, Tomoda et al., reported that maitake (Ganoderma lucidum) had a blood glucose-lowering activity,13 and this activity was examined here in maitake.

First, powdered fruit bodies of maitake were mixed in a commercial feed at the ratio of 1:4 to make a solid feed which was administered to KK-A' mice. These mice were developed with stronger diabetes mellitus by transplantation of the obesity gene A' in to the KK mouse which is spontaneously diabetic. The weight of KK-A' mouse increases as it the animal grows because it has hereditary obesity, and weights of control group mice were always heavier by an average of 10 g than those of the M-feed group, although the N and M-feed groups were given the same amounts (on average, 5.5 g per day). This indicates that fruit bodies of maitake inhibit the weight increase even in mice which have the obesity gene.

Levels of blood glucose, plasma insulin and triglyceride in M-feed mice were all lower with significance of differences compared to those of the control group. These
mice did not have hyperglycemia during the 8 week period on M-feed, indicating the presence of anti-diabetic activity in maitake. However, it is known that diabetic conditions are often improved in NIDDM (non-insulin-dependent diabetes mellitus) due to a reduction of insulin resistance and an improvement in obesity. The NIDDM mechanism can be explained as follows: insulin secretion is increased from pancreas \( \beta \) cells after being stimulated by an excessive intake of energy, resulting in obesity due to the increase of fatty compounds in the body. Obesity would increase the resistance to insulin, causing the high blood glucose. Therefore, the characteristics of NIDDM are high blood glucose, high insulin in blood and high lipid content in blood. Blood glucose of KK-A' mouse, known as the NIDDM model, increases rapidly after 8 weeks when its weight exceeds 35 g, and at the same time the levels of insulin and lipid are high. Because of the relationship between body weight and fatty compounds, obesity caused by the accumulation of fat plays significant role in the high blood glucose. This is why the factor of weight cannot be eliminated in experiments using the KK-A' mouse. The difference of weight in mice between the control and M-feed groups was minimized by feeding 20% M-feed to the N-feed group after the 4th week and feeding N-feed to the M-feed group, to determined movement differences during the final 4 weeks of the experiment. The already-elevated blood glucose level of the mice in N-feed (control) group was reduced to the pre-treatment level during the 1st week, although body weight had not yet decreased. In the same period, the level of blood glucose which had stayed low due to M-feed, increased to 400 mg/dl even during the 1st week of N-feed diet was when no body weight increase had yet been observed. Levels of insulin and triglyceride showed the same tendencies.

These results suggest that the changes in values were caused by the administration of M-feed prior to the change of body weight. They also suggest that maitake is effective in curbing already existing diabetes. An examination of the material causing such biological activity was then made by extracting various fractions from maitake and feeding them individually to KK-A' mice. The blood glucose-lowering activity was observed in the ES and X-fractions; both fractions inhibited the rise in levels of insulin and triglyceride in the blood. No change of body weight was observed by the administration of these fractions.

Tomoda et al. reported that extractable material in mannetake showed a glucose-lowering activity when administration i.p., but this mushroom did not show stronger activity than maitake.

From these results, we came to the conclusion that maitake has anti-diabetic activity which is effective to KK-A' mouse, the model of non-insulin-dependent diabetes mellitus, and that the active agent would be present in the ES and X-fractions which have lipid and polysaccharides as the principal constituents, respectively. Also, it is considered that such effect of maitake is not dependent on the change of body weight in the progress of diabetes, but is directly related to the metabolism since the biological change occurred prior to body weight to change and there was no difference in the body weight among the mice in each fraction group.

Further work to isolate the active agent and elucidate its function and mechanism are now in progress.

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REFERENCES AND NOTES

1) Present address: Department of Microbial Chemistry, Kobe Pharmaceutical University, Motoyama, Higashinada, Kobe 658, Japan.