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Anti-diabetic effects of water extract from the dietary mushroom *Neolentinus lepideus* in type 2 diabetic db/db mice

Ui jeong Yun, Hyesun Jung, Kye Won Park, and Ki-Moon Park*

Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Korea.

ABSTRACT: The objective of this study was to determine the anti-diabetic effect of the water extract of *Neolentinus lepideus* in a diabetic mouse model. Seven-week-old C57BL/KsJ-db/db mice were fed either a control diet (CD) or diet supplemented with 1% or 5% of *N. lepideus* water extract (NLWE1 or NLWE5) for 10 weeks. Oral administration of NLWE significantly decreased the body weight gain compared to that of CD-fed group. Mice in the NLWE group had significantly lower levels of fasting serum glucose, fatty acids, and low-density lipoprotein cholesterol compared to those in the control group. These effects were accompanied by reduced fatty liver and improved glucose tolerance in the NLWE group. Taken together, these results suggest that *N. lepideus* might have potential as a dietary supplement to control diabetes.

KEYWORDS: *Neolentinus lepideus*, Diabetes, Diabetic mouse, Water extract, Oral administration

Introduction

Type 2 diabetes mellitus (DM) characterized by hyperglycemia begins with insulin resistance due to defected insulin secretion in beta cells or failure to respond properly to insulin by peripheral organs (1-3). DM is a leading cause of microvascular and macrovascular diseases. It is also associated with incidents of liver disease, psychiatric illness, arthritis, and cancer (4-6). Although several classes of drugs are available in the market for DM and many are being actively developed, side effects and other disadvantages limit their use in DM (3). Due to increasing incidence of DM worldwide, prevention and alternative therapeutic strategies are mandatory.

Mushrooms have been widely consumed due to their physiological effects and nutritional values (7,8). Mushrooms contain dietary fiber, vitamins, bioactive polyphenolic, and flavonoid compounds. β-glucan, ergosterol, γ-tocopherol, and β-carotene in mushrooms can mediate various biological activities, including prevention against obesity, diabetes, cancer, and cardiovascular diseases (8). Therefore, mushrooms might have potential as alternative treatment for human metabolic diseases. *N. lepideus,* a basidiomycete previously known as *Lentinus lepideus*, is one of the most popular edible mushroom cultured in Japan, China, and Korea (9). It has been reported that *N. lepideus* extracts exhibit antioxidant, antityrosinase, and inhibitory activities on NO production (10,11). *N. lepideus* supplementation can reduce body weight and improve lipid profiles of hypercholesterolemic rats (12). These data suggest that *N. lepideus* supplementation might be useful for other diseases involving metabolic defects.

To determine whether *N. lepideus* might have beneficial effect on metabolic disorders, water extract of *N. lepideus* was fed to diabetic animal models of C57BL/KsJ-db/db (db/db) mice. Our results showed that supplementation of water extracts of *N. lepideus* decreased their body weights, lowered blood glucose levels, reduced fatty liver, and improved glucose tolerance associated with type 2 diabetes. These data suggest that *N. lepideus* might have potential to be developed as functional food or dietary supplement to

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*Corresponding author
E-mail : pkm1001@skku.edu
Tel : +82-31-290-7806, Fax : +82-31-290-7816

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prevent or control type 2 diabetes.

Materials and methods

Preparation of *Neolentinus lepideus* water Extract and high fat diets

Neolentinus lepideus Solhyang was developed and provided by Mushroom Research Institute, Gyeonggi-Do Agricultural Research and Extension Service, Korea. Air dried *N. lepideus* were grounded to powder in a Cyclotec 1093 sample mill (Foss, Seongnam, korea)) and extracted with hot water (70°C) for 72 h at 25°C. The extract was filtered, concentrated with a rotary vacuum evaporator, and freeze dried at -50°C (freeze Dryer, Ilshin Biobase Co. Ltd., Dongducheon, Korea). Compositions of amino acids, sugars, β-glucan, and total polyphenol of the extract have been analyzed previously (13). Diets were supplemented with 1% (NLWE1) or 5% (w/w) of dried extract (NLWE5). The ingredient compositions of experimental diets are shown in Table 1.

Table 1. Composition of the experimental diets (g/100g)

Ingredient (%)	Dietary groups		
	CD[3)]	1% NLWE[3)]	5% NLWE[3)]
Corn starch	39.8	38.8	34.8
Casein	20.0	20.0	20.0
Dexrinized corn starch	13.2	13.2	13.2
Sucrose	10.0	10.0	10.0
Mineral mixture[1)]	3.5	3.5	3.5
Vitamin mixture[2)]	1.0	1.0	1.0
L-cystein	0.3	0.3	0.3
Choline chloride	0.25	0.25	0.25
Tetrabutylhydroquninone	0.0014	0.0014	0.0014
Cellulose	5.0	5.0	5.0
Hot water extracts of NLWE	0.0	1.0	5.0
Soybean oil	7.0	7.0	7.0

[1),2)] AIN-93G mineral and AIN-93G vitamin mixture
[3)] CD; control diets, NLWE; *Neolentinus lepideus* water extract (w/w)

Animal studies

All animal studies were carried out in accordance with the guidelines of the Animal Research Committee (SKKUIACUC-20150037) of Sungkyunkwan University. Seven-week-old C57BL/KsJ-db/db male mice were purchased from Central Lab Animal Inc. (Seoul, Korea). These mice were individually housed in a temperature-controlled room with a 12 h light/dark cycle. After a 1-week adaptation period, eight-week-old mice were randomized into 3 groups and fed a control diet (CD, n =7), a diet supplemented with 1% (w/w) *N. lepideus* powder (NLWE1, n = 7), or a diet supplemented with 5% (w/w) *N. lepideus* powder (NLWE5, n = 7) for 10 weeks. C57BL/6J non-diabetic control mice were also included in the study (n = 7). Diets and water were provided to mice *ad libitum*. They were refreshed three times per week.

For glucose tolerance test (GTT), mice were fasted for 16 h. Tail-vein blood samples at 0, 15, 30, 60, 90, and 120 min after i.p. injection of glucose (1 mg/g) were collected. Glucose levels were determined using GlucoDr^{TM} (All Medicus Co. Ltd., Anyang, Korea) (14). At the end of the experiment, overnight-fasted mice were anesthetized with CO_2 gas and euthanized by cardiac puncture. Blood samples were collected. Sera samples were prepared after centrifugation at 1,000 x g for 15 min at 4°C. They were kept at −70°C until analysis. Livers were harvested, rinsed with 0.9% saline solution, wiped with filter paper, and fixed with 10% paraformaldehyde, dehydrated, and embedded in paraffin. Liver sections with a thickness of 4 μm were subjected to hematoxylin and eosin (H&E) staining.

Serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride (TG) were determined using enzymatic colorimetric kits. Triglyceride E-test kit (Wako Pure Chemical, Osaka, Japan) and Cholesterol E-test Kit (Wako Pure Chemical, Osaka, Japan) were used according to the manufacturer's instructions.

Statistical analysis

Data are presented as means SD. One-way analysis of variance (ANOVA) was performed and significant differences were determined using Student-Newman-Keuls test. Statistical analysis software PASW Statistics 17 (IBM Corp., Somers, NY, USA) was used. Statistical significance was defined as $P < 0.05$.

Results and Discussion

Previous studies have shown that of *N. lepideus* water and ethanol extracts (NLWE) have anti-oxidative and -amylase suppressing activities (13, 15). These data raised the possibility that NLWE might have anti-diabetic effects *in vivo*. To determine the anti-diabetic effect of NLWE *in vivo*, wild type mice (WT) were fed

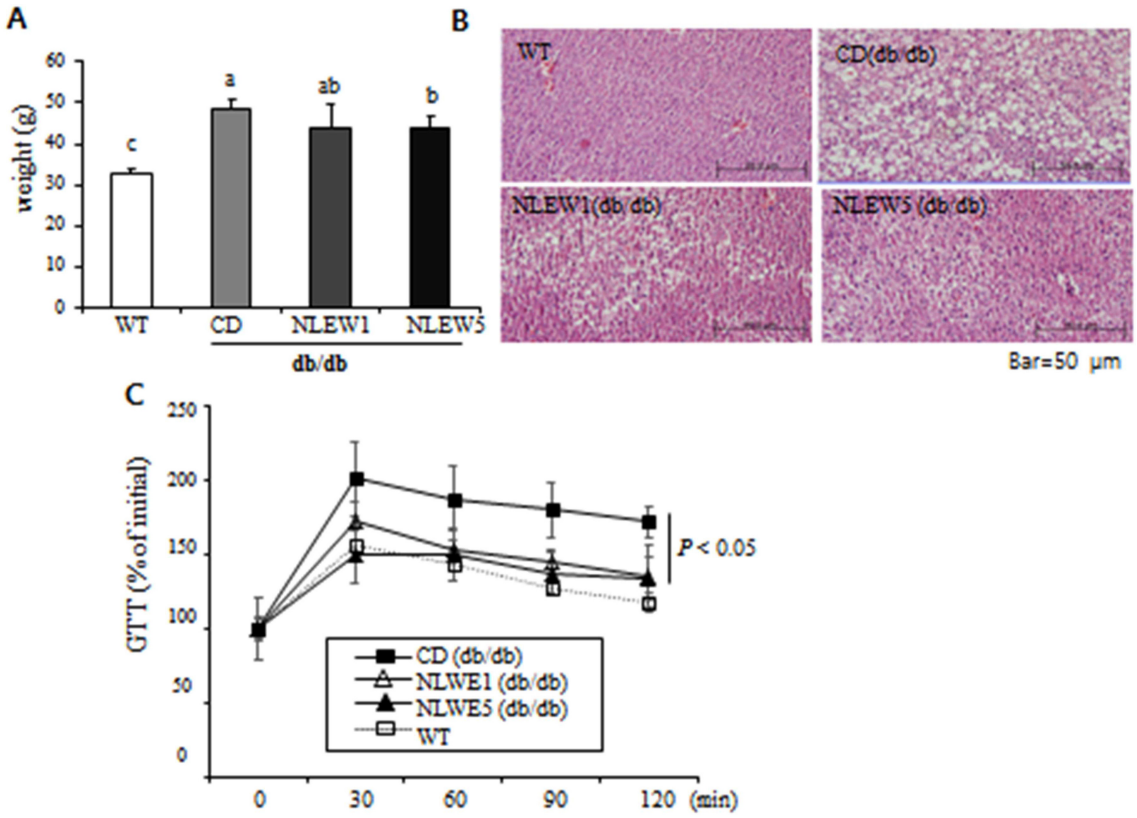

Fig. 1. Anti-diabetic effects of dietary *Neolentinus lepideus* water extracts (NLWE) in db/db mice. C57BL/KsJ-db/db mice were fed a control diet (CD) alone or supplemented with 1% or 5% of water extracts of *Neolentinus lepideus* (NLWE 1 or NLWE5) for 10 weeks. C57BL/6J non-diabetic control mice (WT) fed with CD were used as controls. (A) Body weight gain of control group mice or NLWE-treated mice. Male C57BL/6J mice were fed with a normal diet (WT), C57BL/KsJ-db/db (db/db) mice were fed with diet containing control diet (CD) or NLWE diet (1% and 5%, w/w) for 10 weeks. Data represent means ± SEM (n = 7 per group). Data were analyzed by ANOVA. Different letters indicate statistically significant difference ($P < 0.05$). (B) Representative haematoxylin and eosin (H&E) sections of liver from WT or db/db mice fed with control or NLWE diets. Accumulation of lipid in the liver was decreased in mice fed with NLWE diet. (Scale bar = 50 μm). (C) Significant differences in glucose tolerance test (GTT) in the control and NLWE-administrated groups (n = 7 per group). Tail blood samples were collected at different time points for measurement of blood glucose levels. Data represent means ± SEM. Data were analyzed by ANOVA. Different letters indicate statistically significant difference ($P < 0.05$).

with normal diet while C57BL/KsJ-db/db (db/db) mice were fed with a control normal diet (CD) or diet supplemented with 1% (NLWE1) or 5% (NLWE5) NLWE for 10 weeks (Table 1). After 10 weeks of dietary treatment, NLWE supplementation significantly reduced the body weight of mice without affecting their food intake. The average body weights of mice in the NLWE5 and NLWE1 groups were 5.0 g and 4.7 g lower, respectively, compared to those in the control group (Fig. 1A). However, food intake was similar among the control group (4.73 g/day), NLWE1 group (4.73 g/day), and NLWE5 group (5.06 g/day).

Similar to human diabetes, diabetic db/db mice are associated with increased cholesterol contents and glucose levels (16). We measured the levels of fasting glucose, triglycerides (TG), total cholesterol, HDL- and LDL- cholesterol levels in these mice fed with different diets. As shown in Table 2, serum glucose level was 142 mg/dL in WT mice, 596 mg/dL in the control db/db group, 511 mg/dL in NLWE1 group, and 474 mg/dL in NLWE5 group. Plasma free fatty acid (FFA), total cholesterol, LDL-and HDL- cholesterol levels in NLWE supplemented mice were also significantly lower compared to those in mice fed with the control diet (Table 2).

Excess accumulation of lipid in hepatocytes can raise the risk for type 2 diabetes (17). To show the effect of NLWE on fatty liver associated with diabetes,

Table 2. Effects of dietary *Neolentinus lepideus* water extract (NLWE) on plasma levels of free fatty acid (FFA), total, HDL, LDL-cholesterol, and Triglyceride (TG) in db/db mice[1)]

	WT[2)]	Diabetic groups		
	CD	CD	1%NLWE	5%NLWE
Glucose (mg/dL)	142.50±16.74^{c}	596.29±67.62^{a}	511.57±102.47^{ab}	474±156.37^{b}
FFA (μEq/ml)	3412.71±634.9^{b}	5274.86±1407.24^{a}	4121.57±847.05^{b}	3583.14±1082.88^{b}
Total cholesterol (mg/dL)	112.87±27.59^{b}	225.25±70.01^{a}	199.50±43.62^{a}	163.75±76.37^{ab}
HDL-cholesterol (mg/dL)	108.79±26.13^{c}	195.40±55.07^{a}	181.34±35.62^{ab}	140.84±56.78^{bc}
LDL-cholesterol (mg/dL)	14.53±3.19^{b}	50.31±19.81^{a}	28.24±13.31^{b}	22.17±13.76^{b}
Triglyceride (mg/dL)	121.29±13.73^{b}	190.71±58.33^{a}	153.86±43.05^{ab}	157.57±56.23^{ab}

[1)] Values represent the means +/- SEM. The data were analyzed by ANOVA. The different letters indicate statistically significant difference ($P< 0.05$)

[2)] WT; wild type control (n=5), Diabetic groups; diabetic mice, db/db, CD; control diets (n=5), NLWE1; 1% NLEW diet (n=5), NLWE5; 5% NLEW diet (n=5)

hematoxylin and eosin staining was performed. Increased lipid accumulation was evident in the liver of db/db mice fed with the control diet compared to that in wild type mice. Fatty liver was significantly prevented by administration with 5% NLWE, whereas modest reduction in fatty liver was achieved by supplementation with 1% NLWE (Fig. 1B), further suggesting that NLWE can ameliorate diabetes and liver steatosis.

To further examine whether NLWE could improve glucose homeostasis in diabetic mice, we performed glucose tolerance test. NLWE treated groups showed significantly improved glucose tolerance compared to the CD fed db/db mice (Fig. 1C). These data were consistent with improved blood lipid profiles and reduced fatty liver in mice supplemented with NLWE. Taken together, these data suggest that oral administration of NLWE could improve glucose metabolism and insulin sensitivity in db/db mice.

If translated into humans, NLWE might possess promising anti-diabetic ingredients for preventing type 2 diabetes. Currently, the underlying mechanisms for the beneficial effects of NLWE treatment on diabetes has not been clearly elucidated yet. Body weight reduction, improved fatty liver, and serum lipid profiles may account for its actions on glucose metabolism. It is less likely that energy intake could mediate the effect since food intake was similar among these groups. Alternatively, increased energy expenditure in the NLWE may play a role in its beneficial effects on weight reduction and diabetic symptoms in db/db mice. Increased insulin sensitivity by NLWE may be another axis that can be examined by investigating insulin mediated signaling pathways in the future. In addition, previous studies showed that ethanol extracts from *N. lepideus* exhibited anti-glucosidase activity in streptozotocin-induced type 1 diabetic rat models, suggesting that *N. lepideus* might have beneficial actions against both type 1 and type 2 diabetes (15).

This study had some limitations. We did not measure insulin tolerance tests and activation of signaling components to definitely assess the improved insulin sensitivity by NLWE supplementation. Although the current data support the anti-diabetic effects of NLWE in type 2 diabetic mice, detailed analysis will be necessary to resolve these issues. Another limitation is that the active anti-diabetic components in NLWE were not determined. We have not identified phenolic compounds in the *N. lepideus* extracts, bioactive compounds although their concentrations might be low, but might exhibit effects against metabolic diseases including diabetes (11,13,18). We also cannot exclude the possibility that non-polyphenolic compounds might also contribute to the anti-diabetic actions of *N. lepideus* (19, 20). Further studies are also needed to determine the identities of bioactive constituents contained in NLWE for diabetes.

In conclusion, our results demonstrated that water extract of *N. lepideus* had anti-diabetic effects in diabetic C57BL/KsJ-db/db mice. We found that NLWE improved lipid profiles, lowered blood glucose levels, inhibited body weight increases, prevented fatty liver, and improved glucose metabolism. Taken together, these data suggest that NLWE might have the potential to be developed as functional food against type 2 diabetes.

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