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Review article

Nutraceutical and therapeutic significance of Echigoshirayukidake (*Basidiomycetes-X*), a novel mushroom found in Niigata, Japan.

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Abstract

Mushrooms are recognized as one of the important foods having vital roles in human health, nutrition and medicine. Echigoshirayukidake (*Basidiomycetes-X;* BDM-X) is a mushroom discovered in Niigata, Japan in 1994 and was later identified as a new species belonging to Basidiomycota. Its truffle-like shape comes from the unique property of this mushroom that it does not form mycelium the same as other basidiomycetes family mushrooms such as Shiitake and Agalicus. BDM-X is also characterized by its high content of β-glucan, being as high as 21% (w/w). Since it was artificially cultured, many studies on its physiological and pharmacological effects have been progressed, and a variety of functions have been reported including antioxidant, anti-allergic, anti-inflammatory, anti-obesity, and hepatoprotective functions. A few clinical trials also showed that BDM-X uptake ameliorates atopic dermatitis and prevents liver damage. Antioxidant activity guided isolation revealed the presence of specific formyl pyrrole analogues as the major active ingredient candidate of BDM-X, and among them, 4-(2-formy-5-hydroxymethyl-pyrrole-1-yl)-butanoic acidamide was found to be specific for BDM-X. In this article, we discuss the functional properties of this newly discovered mushroom and possible application as a resource for functional foods and medicines.

***KEY WORDS*:** Echigoshirayukidake (*Basidiomycetes-X*: BDM-X), antioxidant, anti-obesity, anti-atopic function, anti-inflammation, hepatoprotective function.

Introduction

Mushrooms are quite familiar to us nowadays as a food since several mushrooms are now cultivated, and distributed in markets as a popular ingredient to modify daily cuisines. However, they have had a close relation with human life since ancient times, not only for foods but also for medicinal tools and, sometimes, as a tool in ritualistic arts like Shamanism, because they are widely distributed in nature and carry diverse pharmacological functions in spite of being edible or toxic [1].

Metabolic syndromes, and obesity in particular, are gateways to type 2 diabetes, which is now a social issue worldwide [2]. It is well understood that lifestyle, especially diet, is closely related to this disorder and thus the preventive roles of foods are extensively discussed. In this sense, mushrooms are attracting attention as a healthy food fighting against diabetes because they are low in fat and calorie but rich in nutrients such as vitamins and minerals [3]. Moreover, recent studies revealed mushrooms have a wide range of pharmacological and physiological functions contributing to the prevention of diseases including metabolic syndromes, cancer and even dementia [4].

Medicinal functions of mushroom were primarily focused on for their anti-cancer effects and immune modulating activity, and several polysaccharide fractions are

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proposed as the active principle, such as polysaccharide-K (Krestin) from *Trametesversicolor*[5] and Lentinan from *Lentinula edodes* (Shiitake)[6], which have been approved for clinical use to treat cancers. Since precise mechanisms of mushroom's action were not easily understood by the pharmacology based on the ligand-receptor interaction theory, the idea of Biological Response Modifier (BRM) has been proposed as the homeostatic function of mushrooms[7], but now such effects are explainable in part by the immune modulating activity[8]. Since physiological or pharmacological functions of food are currently defined as the third function of foods in addition to the other two essential functions, nutritional and sensory functions, the search for active ingredients in foods (food factors) has been accelerated worldwide[9]. Mushrooms have been the target of such studies and, besides their biological functions as a whole, the search for their active ingredients has progressed for various mushroom species, and thus multiple functions of mushrooms are now implicated as the functions of these active ingredients[10].

Echigoshirayukidake is, in this sense, one of the attractive research targets. This mushroom was originally found in the mountainous region of Uonuma, Niigata, Japan in 1994. The mushroom was primarily used in the limited area as a tea for health promotion and some researchers were also interested in the anticancer activity as a folk medicine. Echigoshirayukidake is now artificially cultured and thus, basic studies are facilitated on the functional properties of this unique mushroom.

In this article, we describe the recent progress of functional studies on this novel fungal resource and discuss the possible application for functional foods against conditions such as metabolic syndromes, liver damage and atopic dermatitis (***Fig. 1***).

Echigoshirayukidake as Bashimidomycetes-X

Echigoshirayukidake was primarily reported as white truffle at the meeting of Japanese Society of Fungi[11], because it has a truffle like appearance when grown on cultivar. However, the precise DNA comparison carried out later revealed Echigoshirayukidake is a new species belonging to Basidiomycetes and was given a name "*Bashidiomycetes-X*" (BDM-X) to register in the database of the NPO organization for International Patent Organism Depositing (IPOD) in the Industrial Technology Institute of Japan (PCT/JP2004/006418) in 1999.

Mushroom is often called fungi, and belongs to the large biological kingdom including not only mushrooms, but also yeast and mold[12]. Recent classification of fungi using morphology and rRNA analysis identified four major phyla which are Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. Among them, the fungi forming fruiting body as the reproductive organ for spore formation are usually called mushroom, but the shapes of mushroom as macrofungi are diverse depending on sporophores conditions[13]. Many of our familiar mushrooms are present in the Ascomycota and Basidiomycota phyla. For example, *Lentinula edodes* (*Shiitake*) and *Flammulina velutipes* (Enokitake) are included in the Basidiomycota to which BDM-X belongs. BDM-X, however, is unique and different from typical mushrooms having an umbrella-shaped fruiting body because it does not form basidium, a spore-releasing organ, and grows in various shapes of sclerotium according to the environment where it grows.

BDM-X is now artificially cultivated in mass scale. Briefly, the mushroom bed medium is prepared by using sawdust of broadleaved trees as raw material and mixing it

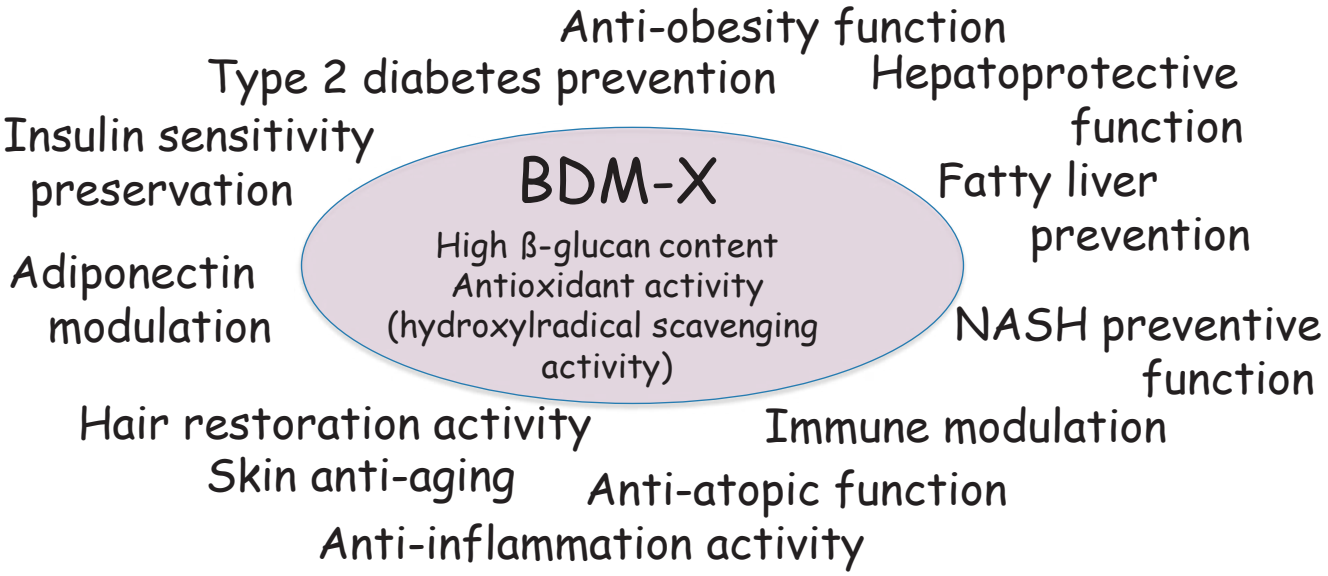

Fig. 1. **Functions studied for *Basidiomycetes-X*.**
BDM-X, *Basidiomycetes-X*.

with food byproducts such as defatted rice bran, wheat bran and mycelia activators as the nutrient source. The cultivation process involves sterilization of the mushroom bed packed in a plastic bag, and after inoculation, BDM-X is cultured for approximately one year in an air-conditioned clean room and then harvested. BDM-X forms a pale pink to white potato-like mycelial mass (sclerotium) with pale snow-like white mycelium centered around the inoculation site when cultured on agar or sawdust medium containing potato glucose as the nutrient (***Fig. 2-A***). The aqueous extract of BDM-X was dried and powdered as in ***Fig 2-B***. The general nutritional composition of dried powder of BDM-X is given in ***Table 1***.

Antioxidant and Anti-inflammation activity of BDM-X

Oxidative stress and inflammation are essentially associated with many chronic diseases and thus anti-oxidant and anti-inflammatory activities are the basic requirement for functional foods[14]. Indeed, the antioxidant activity of mushrooms has also been discussed[15]. Many antioxidant components are contributing to the antioxidant activity of mushrooms such as phenolics, flavonoids, terpenoids, vitamins, ergothioneine, and polysaccharides. Among them, phenolics and polysaccharides are rather common in mushrooms, such

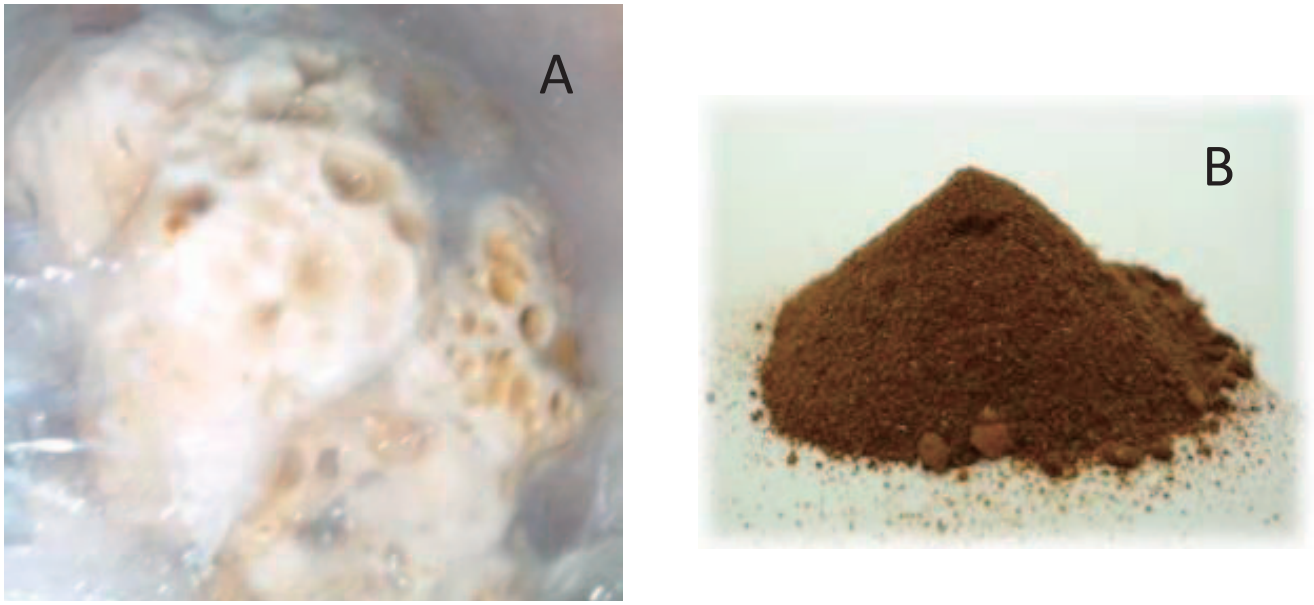

Fig. 2. **Appearances of BDM-X during cultivation (A) and the dried power of extract (B).**
BDM-X, *Basidiomycetes-X*.

Table 1. Nutritional composition of BDM-X.

Item	Unit	BDM-X dry powder (per 100g)
Energy	kcal	179.0
Water	g	8.2
Protein	g	16.0
Fat	g	1.9
Carbohydrate	g	36.7
Dietary fiber	g	32.7
Ash content	g	4.5
Sodium	mg	10.1
β-glucan	g	13.5

BDM-X, *Basidiomycetes-X*.

as low molecular phenolics from *Chaga*[16], and extra-and intra-cellular polysaccharides, respectively, from *Pleurotus geesteranus*[17] and *Pleurotus erringii*[18].

Antioxidant activity of hot water extract of BDM-X was studied by Watanabe *et al*. using several assay systems in that *Agaricus Blazei* Murill (ABM) and lipoic acid were measured as positive reference antioxidants[19]. ABM is an alternate β-glucan rich mushroom and is reported to have several functionalities as herbal medicine[20].

DPPH radical scavenging activity assay of hot water extract of both BDM-X and ABM revealed BDM-X has stronger activity than ABM. Their iron reducing ability showed the same tendency as the DPPH radical scavenging behaviors. These differential antioxidant properties of BDM-X and ABM were reflected *in situ* inhibitory potentials against the lipid peroxidation in both artificial liposome and rat liver homogenate, that was initiated by 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH). It was noted that BDM-X had stronger scavenging activity towards hydroxyl radical than superoxide radical, but ABM showed stronger activity towards superoxide than hydroxyl radical.

The preventive function of BDM-X on *in vivo* liver oxidative damage was further examined using mice. The mice were pre-treated with BDM-X (40 mg BDM-X/mouse) following the injection of lipopolysaccharides to cause liver injury. The results showed both TBARS and nitrotyrosine formations as markers of oxidative stress were more extensively prevented by BDM-X compared to ABM, although ABM showed almost the same inhibitory activity *in vitro* as BDM-X. Moreover, the inhibitory action on nitrotyrosine formation was more markedly shown by BDM-X than ABM. Since nitrotyrosine formation is known as the indication of hydroxyl radical mediated oxidative stress[21], the hydroxyl radical scavenging potential of BDM-X is critically involved in the protective action of BDM-X against liver oxidative damage. It was also found that the antioxidant potential of BDM-X was almost equivalent to or higher than α-lipoic acid. which is considered an ultimate physiological antioxidant[22]. The IC_{50} value (50% inhibition concentration) of BDM-X was approximately 0.53 g dry weight/kg body weight/ day, which roughly corresponds to 16 mmol/kg body weight/day of α-lipoic acid as a reference antioxidant. These results indicated BDM-X has preventive potential against oxidative stress-induced liver damage.

Dextran sulfate sodium (DSS)-induced colitis model is a useful animal model to evaluate the *in vivo* inflammatory potential of natural products[23]. Watanabe *et al*. observed the marked anti-inflammatory potential of BDM-X using this experimental system[24]. In their study, C57BL/6 female mice (7-week old) were fed with drinking water containing 3% DSS for 1 week with and without giving BDM-X (480 mg/kg body weight) orally once a day for 7 days to evaluate the preventive function of BDM-X against colitis formation. Bodyweight and fecal appearance were recorded daily during the feeding periods. After 7 days of BDM-X administration, the gastrointestinal tract as a whole was taken out and the colon length was measured. At the same time, colon sections were histochemically inspected after Hematoxylin-eosin staining and also the expression of inflammation marker proteins was determined. The disease activity index (DAI) score showed the BDM-X administration suppressed the progression of disease condition that was observed in DSS control mice. Inflammation marker proteins such as IL-2Rα expressed in the DSS group were also significantly reduced in BDM-X given mice group, thus indicating BDM-X administration is beneficial to suppress the chronic inflammatory diseases like colitis.

Anti-obesity and metabolic syndromes preventive functions

It is now well understood that obesity is a major risk factor for metabolic syndromes, and thus management of body weight is essentially important[25]. The search for the natural resources having anti-metabolic syndromes function is, therefore, attracting extensive attention. There are several plant resources known historically to have anti-obesity and type-2 diabetes preventive functions such as *Morus alba*[26] and *Salacia oblonga*[27], in that 1-deoxynojirimycin and salacinol, respectively, have been identified as α-glucosidase enzyme inhibitor molecules. Dietary fibers are also the food factor playing important roles in preventing metabolic syndromes including obesity[28], and therefore, anti-obesity and anti-diabetes functions have been studied in several mushrooms or fungi because the dietary fibers are their major component[29].

The anti-obesity effect of BDM-X was precisely studied in animals by Sato *et al*.[30] and reported that BDM-X has remarkably high potential in suppressing obesity compared to the purified β-glucan fraction of *Grifola frondosa* (Maitake). They observed body weight change of male Wistar rats by feeding high fat and high sucrose diet (HFHS) with and without supplementing 5% w/w BDM-X for 100 days. The results showed that, although no difference was observed in the total food intake between the HFHS and HFHS + BX groups during the feeding period, body weight gain observed in BDM-X supplemented HFHS diet was significantly low compared to the reference HFHS diet group, and the level was almost the same as that of the reference normal diet (AIN-93M) fed group. At the same time, visceral fats deposits such as mesenteric, perirenal and epididymal fats were measured. Furthermore, it was found that BDM-X significantly prevented the fat accumulation in these tissues compared to the HFHS diet group. Oral Glucose Tolerance Test (OGTT) was examined for the rats after 100 days feeding trial to know if BDM-X has modulated insulin tolerance acquisition during the feeding period. The results revealed that insulin sensitivity was decreased in the HFHS diet rat group but BDM-X supplemented diet group significantly prevented the decrease. Therefore, modulation of insulin sensitivity is involved in the anti-obesity function of BDM-X.

The anti-obesity function of BDM-X was also studied by Afifa *et al*.[31] using genetically defined obesity model rats (OLETF). In this experiment, OLETF rats which were fed the diet supplemented with 5% BDM-X significantly prevented the body mass gain compared to the rats fed the BDM-X free reference diet. At the same time, body fat accumulation in the visceral tissue also significantly decreased with BDM-X supplemented diet. At the same time, it was noted that BDM-X significantly recovered both the lowered plasma adiponectin level and decreased insulin sensitivity in the obese rats.

Additionally, OGTT was carried out to examine the effect of BDM-X gavage on the postprandial glucose spike

using LETO normal rats, as shown in ***Fig. 3***. BDM-X gavage effectively suppressed the blood glucose spike caused by white rice and glucose gavage, respectively. This glycemic index-lowering effect of BDM-X emphasizes the usefulness of BDM-X in daily meals for controlling blood sugar.

Liver injury protection by BDM-X

At the end of long term feeding trial described above, the fatty liver symptoms were histologically and biochemically observed in the HFHS-diet fed rat group such that the total lipids, TG, and total cholesterol in the liver tissue were significantly increased and the liver damage markers in the blood, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and LDL cholesterol, were also increased. However, in the rats fed BDM-X supplemented diet, these markers were remarkably reduced almost to the level of rats fed normal regular diet, indicating the fatty liver condition occurred by HFHS diet feeding was ameliorated. These imply that the liver damage protection is one of the attractive functions of BDM-X.

Hepatoprotective function has been reported in many edible mushrooms and has been discussed, such as in a review article published in 2013[32]; in the 19 edible mushrooms studied, in various liver damage models are listed, including *Antrodia*, *Lentinula*, *Macrocybe*, *Pleurotus*, *Agaricus*, *Antrodia*, *Panus*, *Calocybe*, *Astraeus*, *Phellinus*, *Coprinus*, *Funalia*, *and Gonaderuma* species. Since 2013 to present (2019), more studies on antioxidant activity and hepatoprotective function of mushrooms are accumulated and some typical examples of edible mushrooms that were not listed in the above review are summarized in ***Table 2***. In these reports, many types of polysaccharides are discussed as antioxidant active principle and the hepatoprotective functions are mainly studied in CCl4 or alcoholic liver damage animal models. However, few studied on non-alcoholic steatohepatitis (NASH), which is liver inflammation and damage caused by a buildup of fatty liver and leads to cirrhosis[33].

Watanabe *et al.*[34] currently studied the protective action of BDM-X on liver damages, including non-alcoholic fatty liver (NAFLS) and NASH in rats which were fed a high fat diet for 16 weeks after streptozotocin (STZ) treatment to produce a NASH condition. Gavage administration of BDM-X (500mg/day) started from the 12th week to see the preventive and recovery functions of BDM-X on NAFLS and NASH. The preventive function of BDM-X was clearly shown by histopathological and biochemical investigations in not only the inflammation marker proteins but fibrosis marker expression which were significantly inhibited in the liver of BDM-X treated rats compared to NASH mice, indicating that BDM-X can inhibit the transition from chronic hepatitis to cirrhosis and even lead to cancer. These results are now under the review process for publication.

Anti-allergic and immune modulating functions

Anti-allergic effect is one of the functions expected for glucan-rich mushrooms as the immune modulator[35], such as *Agaricus blazei*[20]. The high content of β-glucan is one of the characteristics of BDM-X (***Fig. 4***) so that immune regulatory function will be another target of study on BDM-X.

Beneficial use of BDM-X on atopic dermatitis has been clinically examined by uncontrolled open trials on patients

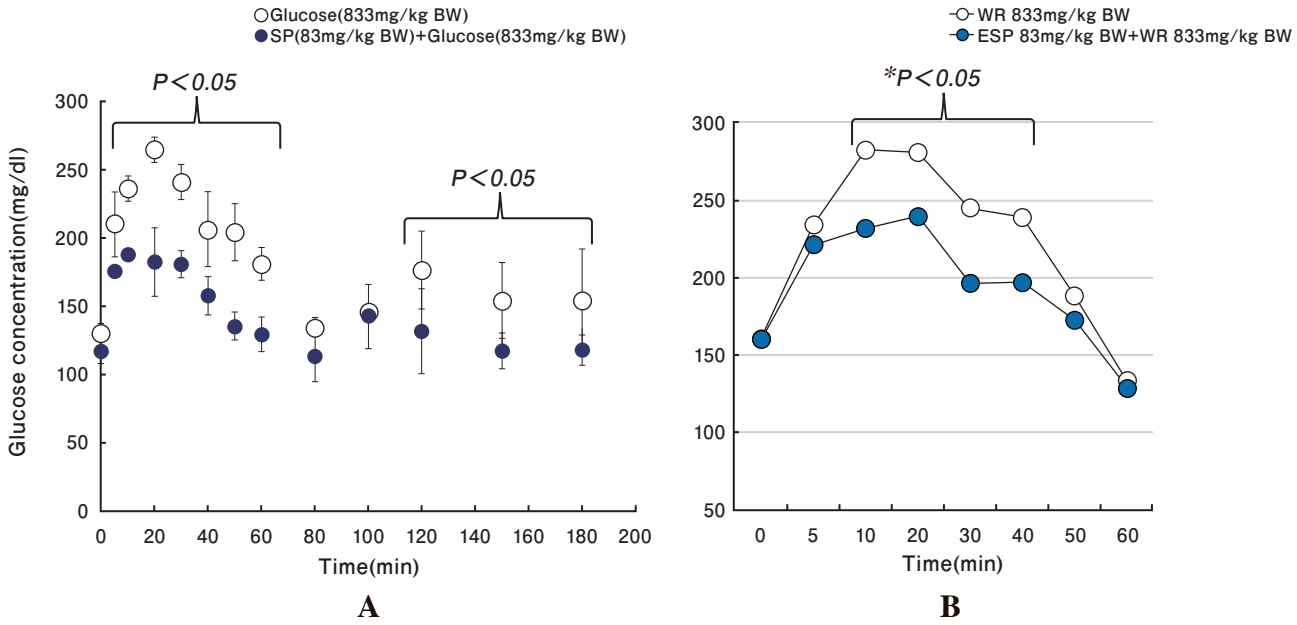

Fig. 3. **Effects of BDM-X on OGTT using glucose standard and white rice as sugar source.**

Data are represented as mean ± SEM (n = 6 /group). Significant differences $p < 0.05$. After gavage administration of standard glucose solution following to BDM-X gavage, the plasma glucose level was monitored **(A)**. Significant differences were observed from 10 to 60 min between glucose and glucose + BDMP groups. At 90 min, the same glucose solution was loaded again. The glucose levels were not increased as high as the first shot but significant difference was also found from the point of 120 to 180 min. In the second test **(B)**, the postprandial plasma glucose rise was examined with white rice powder gavage. BDM-X, *Basidiomycetes-X*; SEM, standard error mean.

Table 2. Edible mushrooms having hepatoprotective function (selected reports during 2014-2019).

Mushroom species	Active ingredients and functions	References
Auricularia auricular	Melanin with molecular weight of 49 KDa. Protection against alcohol-induced liver injury.	Hou R *et al*. (2019) *Food Func* 10,1017
Auricularia polytricha	Two purified monosaccharides. In Hepatoma cells and animal, heaptoprotective and fatty liver preventive functions.	Zao S *et al*. (2019) *Sci Rep* 9, 13725
Coriolus versicolor	Purified Polysaccharide with MW 211.7 KDa. Prevented alcohol induced liver damage in mice.	Wang SK *et al*. (2019) *Int J Biol Macromol* 137, 1102
Basidomycetes-X Echigoshirayukidake	Pyrrole aldehydes, Polysaccharides. Liver damage protection, Prevention of fatty liver injury and STZ-induced NASH.	Present review
Hericium erinaceus	Intra and extracellular polysaccharides. Prevented CCl_4 induced liver damage.	Cui F *et al*. (2016) *Current Microbiol* 73, 379
Hypsizygus marmoreus SK-02	Mycelia Se polysaccharides. Prevention of CCl_4 induced liver damage in animal.	Lin M *et al*. (2016) *Biol Trace Elm Res* 172,437
Laetiporus sulphureus	Hot water and enzymatic extractable polysaccharides. Prevented alcoholic liver damage.	Zhao H *et al*. (2017) *Oxid Med Cell Longev* 2017:5863523
Oudemansiella radicata	Enzyme extracted polysaccharides. Protection against alcohol induced liver damage in mice.	Wang X (2018) *Molecules* 23, 481
Pholiota dinghuensis Bi	Mycelial polysaccharides. Amelioration of CCl_4 induced liver injury in mice.	Gan D *et al*. (2012) *Food Chem Toxicol* 50, 2681
Pholiota nameko SW-02	Mycelial Zn polysaccharides. Preventive function against non-alcoholic fatty liver injury.	Zheng L *et al*. (2014) *Int J Biol Macromol* 70,523
Pleurotus citrinipileatus	Specific Polysaccharides. Ameliorated CCl_4 liver injury, and improved fibrosis.	Lu M *et al*. (2019) *Int J Biol Macromol* 131,315
Pleurotus djamor	Mycelia Zinc polysaccharides. Prevention of CCl_4 induced liver damage.	Zang J *et al*. (2016) *Carbohydr Plym* 136, 588
Pleurotus eryngii SI-04	Exopolysaccharides and its enzyme hydrolysates. Inhibition of hyperlipidemia and attenuation of hepatocyte injury in fatty liver of mice. Intracellular polysaccharides. Preventive effect on acute alcoholic liver disease in animal.	Zang C *et al*. (2017) *BMC Complement Altern Med* 17, 403
Pleurotus eryngii var tuoliensis	Several polysaccharide fractions. Remediate alcoholic hepatitis.	Xu N *et al*. (2017) *Carbohydr Polym* 157, 196
Pleurotus geesteranus	Heteropolysacchride with α-glycoside bond. Reduced detrimental effect of alcohol to liver. Enhanced hepatic enzyme activities.	Song X *et al*. (2018) *Int J Biol Macromol* 114,979
Polyporus unbellatus	Polysaccharides, Effective for treating Hepatitis B.	Liu YM *et al*. (2019) *Prog Mol Bio Transl Sci* 2,284
Russula vinosa Lindblad	Water soluble and alkali soluble polysaccharides. Prevented CCl_4 induced liver damage in animals.	Liu Q *et al*. (2014) *J Agric Food Chem* 62,8858
Termitomyces albuminsus	Polysaccharides, Enhanced antioxidant enzymes via HO-1/Nrf2 pathway to protect CCl_4 induced chronic liver injury.	Zhao H *et al*. (2019) *Int J Mol Sci* 20 (19),

diagnosed with atopic dermatitis (7 men, 15 women; average age 32.1 ± 13.0 years)[36]. Pelleted BDM-X powder (600 mg) was taken once a day for 2 months. During the uptake trial, the patients were allowed to live their usual life without changing their daily lifestyles, such as medication and diet.

For evaluating the therapeutic effect in this study, skin condition was clinically inspected together with biochemical examinations, including liver damage markers. Quality of life (QOL) was evaluated by the graded itching condition. As shown in ***Fig. 5***, the QOL of patients significantly improved during the day as well as night. After two months of uptake of BDM-X tablet, skin redness decreased and skin condition remarkably improved; as much as 73% of the subjects were ranged in satisfactory improvement grade, with 11 subjects (50% of total 22 subjects) showing satisfactory improvement, 5 subjects (23%) showing slightly satisfactory improvement, 4 subjects (18%) showing no change, and 2 subjects (9%) showing unsatisfactory improvement effect. There were no observable adverse effects, such as worsening of symptoms and blood biochemical markers in the subjects that happened during and after BDM-X treating periods. The results suggest that BDM-X may be useful as a therapeutic dietary supplement for patients with atopic dermatitis.

Watanabe *et al*. studied the ameliorative effect of BDM-X on atopic dermatitis also in animal[37]. They examined the effect of BDM-X on atopic dermatitis skin lesions model that was caused by topical application of house dust mite extract following BDM-X administration orally for two weeks. They reported BDM-X attenuated the development of atopic dermatitis in terms of clinical symptoms and associated inflammatory reactions in the skin observed both in histopathological and biochemical examinations, and concluded BDM-X has the potential to inhibit atopic dermatitis through modulating Th1 and Th2 responses and suppression of mast cell infiltration.

Cancer immune modulation activity

In addition, there are several preliminary unpublished data supporting immune modulating function of BDM-X, such that a group of St. Marianna Medical University observed marked increase of plaque-forming cell number in the spleen after injection of hot water extract of BDM-X in mice. Furthermore, in terminal cancer patients, T-lymphocytes ($CD3^+/HLA\text{-}DR^+$) almost doubled after the intake of BDM-X extract for 8 months. These observations are interesting because BDM-X has been used for treating cancer as a folk medicine in localized areas. Although there are similar clinical observations accumulated, such that lymphocytes were significantly increased in terminal cancer patients after taking Shirayukidake extract, more precise and extended studies are required on cancer immune modulating activity and the beneficial use of BDM-X in cancer treatment.

Toxicity

BDM-X was primarily reported as white truffle and thus has longer than 20 years of history as a rare cuisine material served on restaurant menus before precise analysis identified the mushroom as a new Basidiomycetes mushroom, but it was lucky to know there were no recognizable adverse effects such that no toxicity was reported among the people that consumed this mushroom. However, the toxicity evaluation is essential for providing a new resource like BDM-X in spite of using it as a functional food or cuisine material. Acute and sub-chronic toxicity of BDM-X has been precisely evaluated in 2000 by Japan Food Analysis center Ltd and also by Mie University in Wakayama, Japan. Single-dose (2,000 mg/kg) gavage administration of BDM-X aqueous suspension following 14 days observation revealed a LD50 value is no less than 2,000 mg/kg. Sub-chronic toxicity was evaluated by gavage administration of 3,000 mg of BDM-X per day (15.0 g/day/50 kg body weight) for 90 days. This dose was approximately ten times higher than the dose of BDM-X taken as a dietary supplement by humans. During the feeding period, there were no observable abnormalities or fatal effects. Histological inspections of all organs and tissues, and also hematological inspection did not show any recognizable abnormalities. The genotoxicity of DMSO extract of BDMP was also examined using *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537) and *Escherichia coli* WP2 uvrA, and it was found that BDM-X did not show any

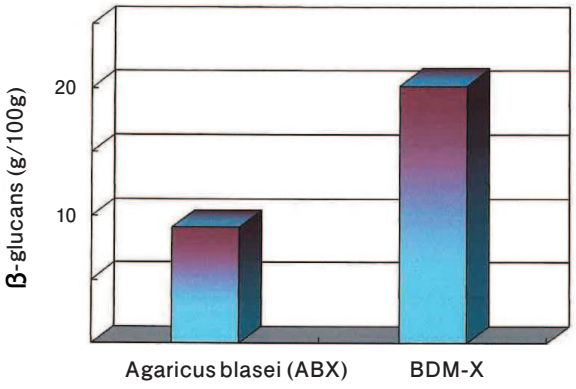

Fig. 4. **Comparison of β-glucans content in BM-X and ABX.**

BDM-X. *Basidiomycetes-X*; ABX, *Agaricus blazei*.

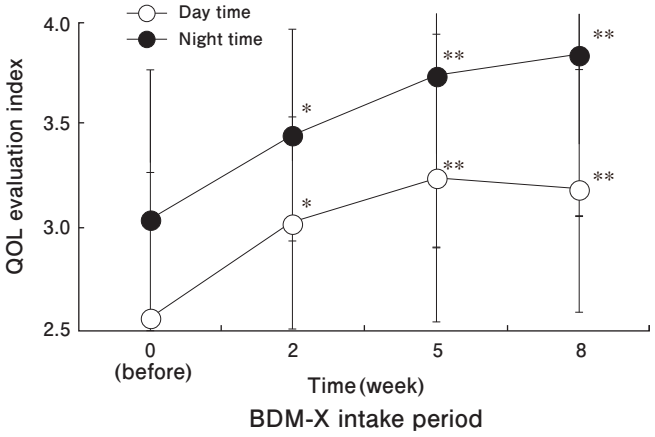

Fig. 5. **Effect of BDM-X uptake on the subjective QOL evaluation index in the patients with atopic dermatitis.**

QOL, quality of life; BDM-X. *Basidiomycetes-X*.

observable mutagenic effect up to 50 mg/mL concentration examined. Taken all together, it was concluded that the toxicity of BDM-X is quite low and thus is essentially a safe food.

The low toxicity of BDM-X was supported by human trial on the ameliorating effects on atopic dermatitis described above[35], where uptake of 600 mg dried powder per day for two months did not give rise to significant adverse effects on either dermatological or blood biochemical markers, including liver damage.

Quite recently, a double blind simple comparison human study was carried out for setting an appropriate dose for supplementation of BDM-X with three different doses of BDM-X (30, 300 and 1,500 mg/day). A total of 48 male and female subjects of age between 40-65 and BMI ranging between 23-30 kg/m^2 were divided into four groups to take tablets containing different amounts of BDM-X or placebo for 12 weeks and to monitor the changes of vital indices such as body weight and fat accumulation, hematological and biochemical indices. In the results, no observable adverse effect was determined even with the highest dose. Moreover, a significant amelioration effect of BDM-X on liver condition was observed, such as the reduction of liver damage markers including γ-GTP, although the dose response was yet unclear[38].

Pyrolaldehyde analogues as expected active principle of BDM-X

As we mentioned above, a wide variety of physiological or pharmacological functions characterize the features of mushrooms or fungi. For examples, Ganoderma[39] and Chaga[40] are the examples of most extensively studied for functions such as anti-cancer, hypoglycemic, hypolipidemic and anti-aging. In these mushrooms, not only glucans, typically β-glucans, but also other low molecular weight metabolites are identified as the active principles of mushroom. For example, Ganoderic acid, a triterpenoid ingredient, found in *Ganoderma lucidum* having hepatoprotective function[41] and a propanoid named BDL (3,4-dihydroxybenzalacetone) from Chaga having neuroprotective function[42]. However, they are wood rotting fungi and are not edible. The edible mushrooms must be more attractive in terms of their beneficial health use in daily life. Currently, many edible mushrooms are artificially cultivated and thus basic studies on their physiological functions are allowed to progress, those include *Lentinus*, *Auricularia*, *Grifola*, *Flammulina*, *Pleurotus*, and *Tremella* species. Anti-obesity and anti-metabolic syndrome functions are the common basic functions of these mushrooms[29]. Many bioactive glucans including β-glucan and many types of heteroglucans are reported as containing the same functional principles as inedible mushrooms. Moreover, several low molecular ingredients are currently attracting much attention, such as elitadenin having hypocholesterolemic function from Shiitake (*Lentinus*)[43]. In *H. erinaceum*, low molecular NGF modulating compounds named Hericenones and erinacine were isolated as the active principles[44], supporting the observations of some small-scale clinical studies showing significant improvement of dementia[45]. *H. erinaceum* has been known as one of three famous rare cuisine ingredients served in the Palace in China together with swallow' nest and bear's hand. At the same time, this mushroom has numerous physiological functions such as anti-gastritis, anti-cancer and anti-aging activities[46]. These suggest that the low molecular secondary metabolites are another target of functional study of mushroom besides the polysaccharides having anti-metabolic syndromes.

An anti-oxidant activity guided search of the active ingredient of BDM-X was carried out by Matsugo's group in Kanazawa university and three carbonyl pyrrolealdehyde analogues (**I**, **II**, **III**) were identified (***Fig. 6***)[47]. Two of them, **I** (2-formy-5-hydroxymethyl-pyrrole and **II** (4-(2-formy-5-hydroxymethyl-pyrrole-1-yl)-butanoic acid), were already isolated from *Morus alba* fruit[48] as the factors activating macrophages. **II** is also found in *Leccinum extremiorientale* and was reported to have hepatoprotective function[49]. The third compound that is the amide analogue of **II** was newly identified as specific in BDM-X. Quite recently, Wood *et al.* reviewed the origin, bioactivity and synthesis of 2-formylpyrrole natural products[50]. They are distributed widely in nature from plants to fungi and are reported to have a range of valuable bioactivities such as hepatoprotection,

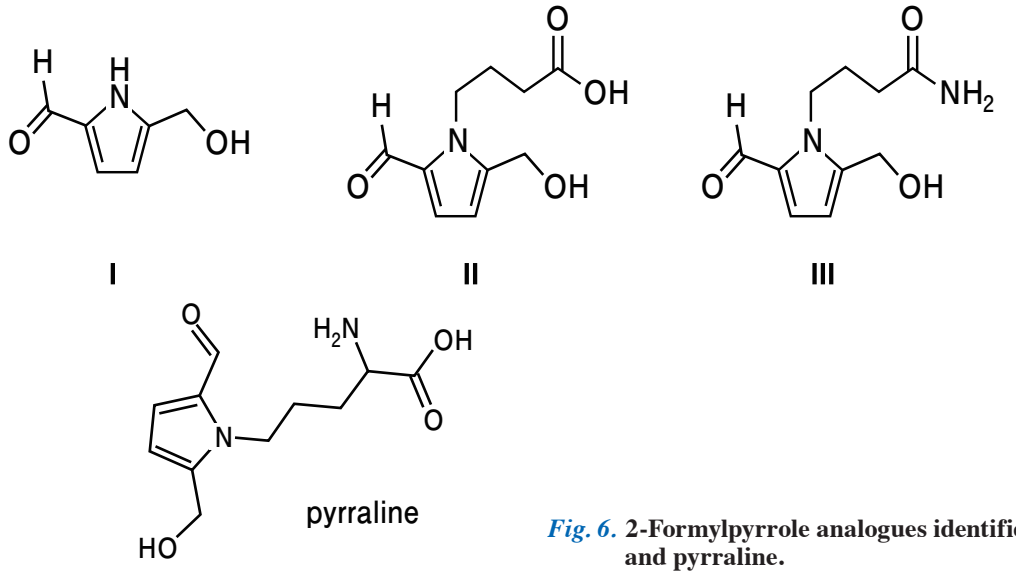

Fig. 6. **2-Formylpyrrole analogues identified in BDM-X, and pyrraline.**

BDM-X. *Basidiomycetes-X*.

immune-modulation, anti-proliferation and antioxidant. It is thus highly plausible that the pyrrole aldehydes in BDN-X are also playing critical roles in the biological and pharmacological functions of BDM-X described above. It is interesting that among the 2-formylpyrroles, pyrraline that is N-lysine analogue of **I** is known as one of the physiological advanced glycation end products (AGE) and implicated as a marker of glycation stress[51]. These 2-hormylpyrrole ingredients, therefore, may possibly act as a ligand modulating AGE receptor mediated physiological reactions. Further, 2-formylpyrroles are expected to be produced by non-enzymatic process and thus are not the secondary metabolite. Therefore, we are waiting for more studies to know how these ingredients are produced in BDM-X and how they are involved in the wide range of physiological and pharmacological functions.

Conclusion

Current progress in the functional studies on mushrooms revealed that mushrooms are an attractive target of study for searching not only bioactive ingredients but also new medicinal functions beneficial for human health and wellness. However, the fungi world is extremely wide and only limited numbers of fungi have been extensively studied so far. Therefore, the search for new species of mushrooms will be important in addition to functional research. Echigoshirayukidake (BDM-X) is one of the examples to support such a stream. BDM-X with a characteristic strange for Basidiomycota was revealed to have a wide variety of physiological and pharmacological functions as other medicinal mushrooms such as *Gonaderma*, *Chaga* and *Agaricus*. The identified unique formylpyrrole ingredients opened the way for further mechanistic studies on these functions, and also applications in functional foods and medicines. Quite recently, Wakame *et al*. identified total 472 different components (368 hydrophilic and 104 lipophilic compounds) in BDM-X extract by the metabolomics approach, which includes aminoacids, lipids, nucleic acid related compounds, organic acids, sugar, polyphenols, and peptides, together with phyrrol analogues described above. This information will be valuable for understanding the physiological functions of BDM-X as food. At the same time, they showed the hair restoration activity and elastase enzyme inhibitory function of the BDM-X extract[52]. Therefore, BDM-X also has a potential as the functional resource for cosmetics. Several drinks, tablet and capsule type products containing BDM-X as the major component are already in the market. Feedback information from users must be another source for collecting supporting evidence for the beneficial role of BDM-X in human health and wellness. Thus, these approaches are also promising.

Conflicts of interest

There are no conflicts to declare.

Reference

1) Wasser SP. Medicinal mushroom science: Current perspectives, advances, evidences and challenges. Biomed J. 2014; 37: 345-356.
2) Fonseca VA. The metabolic syndrome, hyperlipidemia, and insulin resistance. Clin Cornerstone. 2005; 7: 61-72.
3) Rapior S, Hyde KD, Bahkali A. Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Diversity. 2012; 56: 1-29.
4) Smith JE, Rowan NJ, Sullivan R. Medicinal mushrooms: A rapidly developing area of biotechnology for cancer therapy and other bioactivities. Biotechnology Letters. 2002; 24: 1839-1845.
5) Tsukagoshi S, Hashimoto Y. Fujii G, et al. Krestin (PSK). Cancer Treat Rev. 1984; 11: 131-155.
6) Mah-Lee NG, Ann-Teck Y. Inhibition of human colon cancinoma development by lentinan from shiitake mushrooms (*Lentinus edodes*). J Altern Compl Med. 2004; 8: 581-589.
7) Gallagher M, Quinones K. Cervantes-Castaneda RA, et al. Biological response modifier therapy for refractory childhood uveitis. Br J Ophthalmol. 2007; 91: 1341-1344.
8) Volman JJ, Ramakers JD, Plat J. Dietary modulation of immune function by β-glucans. Physiol Behav. 2008; 94: 276-284.
9) Ghosh D, Bagchi D, Konishi T (ed). Clinical Aspects of Functional Foods and Nutraceuticals, 1st ed. CRC Press, Boca Raton, Florida, 2014.
10) Chaturvedi VK, Agarwal S, Gupta KK, et al. Medicinal mushroom: Boon for therapeutic applications. 3 Biotech. 2018; 8: 334.
11) Arai Y, Sawabe A, Morita M. Variation of volatile components by growth of fruiting body of artificially cultivated white truffles, and by their cooking. The 9th Meeting of Japanese Society of Mushroom Science and Biotechnology, August 24-26, 2005, Hiroshima. (abstract in Japanese)
12) Fulton A. Why mushrooms rule the fungi kingdom. Published April 19, 2016. National Geographic. https://www.nationalgeographic.com/culture/food/the-plate/2016/04/19/why-mushrooms-rule-the-fungi-kingdom/
13) Rahi DK, Malik D. Diversity of mushrooms and their metabolites of nutraceutical and therapeutic significance. J Mycolo. 2016; 2016: 7654123.
14) Liguori L, Russo G, Curcio F, et al. Oxidative stress, aging and diseases. Clin Interv Aging. 2018; 13: 757-772.
15) Kozarski M, Klaus A, Jakovljevic D, et al. Antioxidants of edible mushrooms. Molecules. 2015; 20: 19489-19525.
16) Nakajima Y, Sato Y, Konishi T. Antioxidant small phenolic ingredients in *Inonotus obliquus* (persoon) pilat (Chaga). Chem Pharm Bull. 2007; 55: 1222-1226.
17) Song X, Liu Z, Zhang J, et al. Anti-inflammatory and hepatoprotective effects of exopolyssaccharides isolated from *Pleurotus geesteranus* on alchol-induced liver injury. Sci Rep. 2018; 8, 10493.

18) Zhang C, Li S, Zhang J, et al, Antioxidant and hepatoprotective activities of intracellular polysaccharides from *Pleurotus eryngii* SI-04. Int J Biol Macromol. 2016; 91: 568-577.
19) Watanabe T, Nakajima Y, Konishi T. *In vitro* and *in vivo* antioxidant activity of hot water extract of *Basidiomycetes-X*, newly identified edible fungus. Biol Pharm Bull. 2008; 31: 111-117.
20) Firenzuoli F, Gori L, Lombardo G. The medicinal mushroom *Agaricus blazei* Murrill: Review of literature and pharmacotoxicological problems. Evid Based Complement Alternat Med. 2008; 5: 3-15.
21) Huggins TG, Wells-KnechtMC, DetorieNA, et al. Formation of o-tyrosine and dityrosine in proteins during radiolytic and metal-catalyzed oxidation. J Biol Chem. 1993; 268: 12341-12347.
22) Packer L, Witt EH, Tritschler HJ. α-Lipoic acid as a biological antioxidant. Free Radic Biol Med. 1995; 19: 227-250.
23) Chassaing B, Aitken JD, Malleshappa M, et al. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol. 2014; 104: 15.25.1-15.25.14.
24) Watanabe K. *Basidiomycetes-X* ingestion has beneficial role against colitis. The 4th annual meeting of the Society for Echigoshirayukidake research. October 17,2015, Tokyo. (abstract in Japanese)
25) Fruh SM. Obesity: Risk factors, complications, and strategies for sustainable long-term weight management. J Am Assoc Nurse Pract. 2017; 29: S3-S14.
26) Tian S, Tang M, Zhao B. Current anti-diabetes mechanisms and clinical trials using Morus alba L. J Trad Chin Med Sci. 2016; 3: 3-8.
27) Muraoka O, Morikawa T, Miyake S, et al. Quantitative determination of potent alpha-glucosidase inhibitors, salacinol and kotalanol, in Salacia species using liquid chromatography-mass spectrometry. J Pharam Biomed Anal. 2010; 52: 770-773.
28) Cruz-Requena M, Aguilar-Gonzalez CN, Prado-Barragan LA, et al. Dietary fiber: An ingredient against obesity. Emerates J Food Agricul. 2016; 28: 522-530.
29) Ganesan K, Xu B. Anti-obesity effects of medicinal and edible mushrooms. Molecules. 2018; 23: 2880.
30) Sato S, Sakurai M, Konishi T, et al. Anti-obesity effect of Echigoshirayukidake (*Basidiomytetes-X*) in rats. Glycative Stress Res. 2019; 6: 198-211.
31) Khatun A, Sato S, Konishi T. Novel edible mushroom, *Basidiomycetes-X* (Echigoshirayukidake) havin anti-obesity function through manipulation of insulin resistance and lipid metabolism. J Compliment Trad Med. (submitted)
32) Soares AA, de Sá-Nakanishi AB, Bracht A, et al. Hepatoprotective effects of mushrooms. Molecules. 2013; 18: 7609-7630.
33) Stanford Health Care. Nonalcoholic steatohepatitis (NASH). https://stanfordhealthcare.org/medical-conditions/liver-kidneys- and-urinary-system/nonalcoholic-steatohepatitis-nash.html (accessed at November 28, 2019)
34) Watanabe K, et al, Basidomycetes-X ingestion has beneficial role against non-alcoholic steatohepatitis (NASH) in mice, 2019, (under reviewing process for publication).
35) Merdiven S, Lindequist U. Medicinal mushrooms with anti-allergic activities. In; Medicinal Plants and Fungi: Recent Advances in Research and Development, Agrawal DC, Tsay H-S, Shyur, L-F, et al (ed), pp.93-110, Springer, New York, 2017.
36) Minami K, Watanabe T, Yukami S, et al. Clinical trials of *Basidiomycetes-X* (FERMP-19241) on the patients with atopic dermatitis. Medicine and Biology (Igaku to Seibutsugaku). 2007; 151; 306-311. (in Japanese)
37) Watanabe K, Karuppagounder V, Sreedhar R, et al. *Basidiomycetes-X*, an edible mushroom, alleviates the development of atopic dermatitis in NC/Nga mouse model. Experi Mol Pathol. 2018; 105: 322-327.
38) Yonei Y. Report on survey of appropriate dose setting for Echigoshirayukidake use in human. Anti-Aging Bank Ltd, Report No. HR-2018-MT01, 2019. (in Japanese)
39) Khatian N, Aslam M. A review of *Ganoderma lucidum* (Reishi): A miraculous medicinal mushroom. Inventi Rapid: Ethnopharamacology. 2018; 2018: 1-6.
40) Balandaykin ME, Zmitrovich IV. Review on Chaga medicinal mushroom, *Inonotus oblicuus* (Higher Basidiomycetes): Realm of medicinal applications and approaches on estimating its resource potential. Int J Med Mushrooms. 2015; 17: 95-104.
41) Tang YJ, Zhong JJ. Fed-batch fermentation of *Ganoderma Lucidium* for hyperproduction of polysaccharides and ganoderic acid. Enzy Microbial Technol, 2002; 31: 20-28.
42) Gunjima K, Tomiyama R, Takakura K, et al. 3,4-Dihydroxybenzalacetone protects against Parkinson's disease-related neurotoxin 6-OHDA through Akt/Nrf2/glutathione pathway. J Cell Biochem. 2014; 115: 151-160.
43) Tokuda S, Suzuki S, Kanaeda T, et al. Reducing mechanism of plasm choresterol by sii-ta-ke mushroom (IV) : Effect of mushroom on cholesterol metabolism in rats (Part X). Journal of Japanese Society of Food and Nutrition (Eiyo to Shokuryou). 1976; 29: 95-101. (in Japanese),
44) Ma BJ, Shen JW, Yu HY, et al. Hericenones and erinacines: Stimulators of nerve growth factor (NGF) biosynthesis in *Hericium erinaceus*. Int J Fungal Biol. 2010; 1: 92-98.
45) Spelman K, Sutherland E, Bagade A. Neurological activity of Lion's mane (*Hericium erinaceus*). J Restorat Med. 2017; 6: 19-26.
46) Wang M, Gao Y, Xu D, et al. *Helicium erenaceus* (Yamabushitake): A unique resource for developing functional foods and medicines. Food Funct. 2014; 5: 3055-3064.
47) Nishida A, Sakamoto T, Wada N, et al. Structural determination of pyrrole components in *Basidomycetes-X*, a novel mushroom found in Niigata, 2018 (Patent 2017-229598). The 17th Meeting of the Molecular and Environmental Medicine Society, February 2-3, 2018, Tsu, Mie. (abstract in Japanese)
48) Kim SB, Chang BY, Jo YH, et al. Macrophage activating activity of pyrrole alkaloids from *Morus alba* fruits. J Ethnopharmacol. 2013; 145: 393-396.
49) Chin YW, Lim SW, Kim SH, et al. Hepatoprotective pyrrole derivatives of *Lycium chinense* fruits. Bioorg Med Chem Lett. 2003; 13: 79-81.
50) Wood JM, Furkert DP, Brimble MA. 2-Formylpyrrole natural products: Origin, structural diversity, bioactivity and synthesis. Nat Prod Rep. 2019; 36: 289-306.
51) Li H, Yu SJ. Review of pentosidine and pyrraline in food and chemical models: Formation, potential risks and determination. J Sci Food Agric. 2018; 98: 3225-3233.
52) Wakame K, Komatsu K, Konishi T, et al. Metabolomic analysis and the hair restoration activity of BDM-X extract. The 140th Annual Meeting of The Pharmaceutical Society of Japan, March 25-28, 2020, Kyoto. (abstract in Japanese, in press)