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Original article

Effect and safety of Echigoshirayukidake (*Basidiomycetes-X*) on fatty liver: Stratified randomized, double-blind, parallel-group comparison study and safety evaluation study

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Abstract

***Purpose*:** Stratified randomized, double-blind, parallel-group comparison and safety evaluation studies were conducted to verify the efficacy and safety of Echigoshirayukidake (Basidiomycetes-X: BX) dry powder on fatty liver.

***Method*:** Forty-eight of 114 healthy participants (24 males and 24 females) with the highest body mass index, body fat percentage, cholinesterase (ChE), and triglyceride levels were divided into 4 dietary groups (12 each): BX-L, food mixed with 30 mg BX dry powder; BX-N, food mixed with 300 mg BX dry powder; BX-5, food mixed with 1,500 mg BX dry powder; and control, food without BX. Each group consumed the respective food for 6 weeks. Subjects completed the Anti-Aging QOL Common Questionnaire and received a physical examination and blood biochemistry test before and at week 6 and 12 after consumption.

***Results*:** The BX-N group showed a significant decrease in aspartate aminotransferase (AST) levels 12 weeks after (22.0 ± 1.7 U/L) compared to before consumption (24.8 ± 1.5 U/L) ($p = 0.015$), and a significant decrease in lactate dehydrogenase levels 6 (185.1 ± 9.3 U/L) and 12 weeks after (173.8 ± 10.5 U/L) compared to before consumption (195.3 ± 10.9 U/L) ($p = 0.007$, $p < 0.001$, respectively). AST ($p = 0.031$) was significantly improved in the BX-N than the control group. No significant differences were observed in the questionnaire score or γ-glutamyl transpeptidase, alkaline phosphatase, and ChE levels. No BX-induced adverse events were observed in the BX-5 group

***Conclusion*:** Consumption of BX was safe even at 5 times the usual dose. BX dry powder may be a useful functional food component for improving liver dysfunction caused by fatty liver.

***KEY WORDS*:** Echigoshirayukidake (*Basidiomycetes-X*), fatty liver, liver function, antioxidant

Introduction

Echigoshirayukidake (*Basidiomycetes-X*: BX) is a rare new species of edible mushroom discovered in the Uonuma region of Niigata Prefecture in 1994. Dr. Katsuji Yamanaka from the Kyoto Mycological Institute showed that BX was a new species of basidiomycetes (mushroom) that does not form a basidium, and deposited BX into the International Patent Organism Depositary of the National Institute of Technology and Evaluation under the name Echigoshirayukidake (*Basidiomycetes-X*) [1)]. In 2003, a cultivation method for BX using mushroom beds was established for industrial use, enabling distribution to the market. BX is rich in dietary fiber including β-glucan, a polysaccharide, with 100 g of BX dry powder containing 32.7 g of dietary fiber, which includes 13.5 g of β-glucan.

A significant feature of BX powder is its powerful antioxidant effect, which oxidizes biological lipids to promote the formation of lipid peroxides and eliminates hydroxyl radicals that increase the risk of arteriosclerosis [2)]. Studies using model mice have shown that BX powder suppresses the formation of lipid peroxides in the blood and liver [3)]. Additionally, anti-obesity studies using obese rats have shown that body weight gain, lipid metabolism

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disorders and fatty liver are significantly improved in obese rats fed food mixed with BX dry powder compared to food lacking BX dry powder [4, 5]. Therefore, BX is expected to be effective for preventing lifestyle diseases and non-alcoholic fatty liver. A study showed that BX contains 4-(2-formy-5-hydroxymethyl-pyrrole-1-yl)-butanoic acid, which is involved in these actions [1].

We conducted a stratified randomized, double-blind, parallel-group comparison study and safety evaluation study to evaluate the effect and safety of BX in healthy individuals with high body mass index (BMI), body fat, cholinesterase (ChE) and triglyceride (TG) levels.

Methods

Study population

Males and females between the ages of 40 and 65 years at the time that informed consent was obtained were considered for this study. Individuals who had registered with the clinical trial volunteer association were interviewed and recruited over the telephone, and 114 individuals who qualified as subjects of the study underwent screening tests and pre-consumption tests (SCR & Visit-1). Subjects who met the selection criteria, did not satisfy the exclusion criteria described below, and determined to be suitable to participate in the study based on the judgment of the principal investigator were ranked based on their BMI, body fat percentage, ChE and TG levels. The 48 individuals (24 males and 24 females) with the highest values for these parameters were selected as subjects of this study.

Exclusion criteria were as follows:

1) Individuals currently receiving drug therapy for any disease
2) Individuals with a history of mental disorders, sleep disorders, hypertension, diabetes, dyslipidemia or other severe disorders
3) Individuals receiving medication to treat diseases in the past month (excluding single doses for headache, menstrual pain and the common cold)
4) Individuals with a history of severe diseases of the liver, kidney, heart, lung or blood
5) Individuals with serious concomitant diseases and history of diseases of the digestive organs (individuals with a history of major surgery of the gastrointestinal tract such as gastroduodenal ulcer, irritable bowel syndrome, gastrectomy, gastroenterostomy and intestinal resection)
6) Individuals who have donated more than 200 mL of blood in the past month or more than 400 mL within the past 3 months
7) Individuals who have felt ill or experienced poor physical condition after blood collection in the past
8) Individuals with severe anemia
9) Individuals who might develop allergic symptoms to the study diet and who might develop severe allergic symptoms to other foods and medications
10) Individuals whose average daily alcohol consumption, based on the average alcohol consumption, exceeds 40 g/day (2 x 500 mL bottles of beer or 2 bottles of sake)
11) Individuals who smoke over 20 cigarettes a day on average
12) Individuals who might change their lifestyle during the study period (such as those taking long trips)
13) Individuals who are pregnant, nursing or may become pregnant
14) Individuals who are currently participating in other human clinical trials, and those who have yet to reach 3 months since joining other human clinical trials
15) Individuals who themselves or their family members work in companies that develop, manufacture or sell health and functional foods, and cosmetics
16) Individuals who were judged unsuitable as subjects of this study by the principal investigator

The subjects were asked to comply with the following as precautions during the study period: 1) avoid overeating, extreme exercise and lack of sleep; 2) refrain from eating from 12 hours before blood collection up to completion of the test (drinking water is allowed); 3) abstain from drinking alcohol from the day before the test until the end of the study; 4) refrain from changing lifestyle; and 5) refrain from consuming new health foods.

Test food

Four diets were studied in this study: food containing a standard dose (50 mg) of BX dry powder per tablet; food containing a low dose (5 mg) per tablet equivalent to one-tenth of the standard dose to confirm efficacy; food containing a 5-fold dose (250 mg) per tablet to establish safety; and food without any BX dry powder as placebo for comparison with each of the BX diets. Each subject consumed 6 tablets of the assigned study diet per day. Therefore, the test subjects consumed 30, 300, or 1,500 mg of BX powder (low dose, standard dose, 5-fold dose, respectively) daily throughout the study period.

Composition of a single dose and the nutrient composition are shown in ***Tables 1*** and ***2***, respectively. Mycology Techno Co., Ltd provided the test food and control products.

Study design

This was a stratified randomized, double-blind, parallel-group comparison study and safety evaluation study with a placebo control. The stratification factors were age, sex, and BMI. The person responsible for allocation randomly divided the 48 selected individuals into 4 groups: BX low dose blended food group (BX-L), BX standard dose blended food group (BX-N), BX five-fold dose blended food group (BX-5), and placebo food group (control). The allocation table was sealed and kept until unblinding. Consumption of the test food was studied for 12 weeks, and primary endpoints were examined before consumption (week 0), at week 6, and at the end of consumption (week 12). Subjects completed the Anti-Aging QOL Common Questionnaire, and analysis of safety endpoints was conducted before consumption (week 0) and at the end of consumption (week 12) [6, 7]. The subjects maintained a daily diary during the consumption period. The study period was from August to December 2018.

Table 1. Composition of a single dose

Component	BX-L	BX-N	BX-5	Control
Basidiomycetes-X (BX)	5.0	50.0	250.0	–
Food color	–	–	–	15.0
Crystalline cellulose	30.0	30.0	30.0	30.0
Lactose	108.1	93.1	13.1	120.0
Dextrin	150.0	120.0	–	128.1
Hydroxypropyl cellulose (HPC)	0.9	0.9	0.9	0.9
Calcium stearate	3.0	3.0	3.0	3.0
Silicon dioxide (fine particle)	3.0	3.0	3.0	3.0

Values indicate mg

Table 2. Nutrient composition per 100 g.

Nutrient	BX-L	BX-N	BX-5	Control
Energy (kcal)	287	290	292	300
Protein (g)	0.9	1.8	7.8	1.1
Fat (g)	0.5	0.5	0.8	0.6
Carbohydrate (g) (DF + others)	69.7	69.6	63.4	72.6
Na (mg)	14.0	14.0	17.0	42.0

DF, dietary fiber. Others include sugar and starch.

Primary endpoints

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and ChE before and after consumption were used as primary endpoints. Anti-Aging QOL Common Questionnaire [6, 7] scores were used to evaluate subjective symptoms.

Safety endpoints

Blood pressure/pulse, body weight/body fat percentage/BMI, hematology tests, blood biochemistry, urinalysis, and adverse events were determined through medical interviews before and after consumption in the BX-L, BX-N, and BX-5 groups. Hematology test parameters included leukocyte count, red blood cell (RBC) count, hemoglobin, hematocrit, RBC indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration), and platelet count. Blood biochemistry parameters included total protein (TP), albumin (ALB), urea nitrogen (BUN), creatinine (CRE), total cholesterol (TC), TG, low-density lipoprotein-C, high-density lipoprotein-C, fasting plasma glucose, and hemoglobin A1c [National Glycohemoglobin Standardization Program: NGSP]. Urinalysis parameters included occult blood test, qualitative glucose level and protein.

Physical measurements included height, weight, blood pressure (systolic/diastolic) and pulse rate. The body composition test was performed using the RD-503 body composition analyzer (Tanita Corporation, Itabashi-ku, Tokyo, Japan).

The occurrence of adverse events was determined through medical interviews. The principal investigator determined whether the subjects' subjective symptoms, objective findings, and abnormal changes in urinalysis and laboratory values were adverse events.

Statistical analysis

The subjects' background information was expressed as mean ± standard deviation, and the endpoints were expressed as mean ± standard error. Statistical analysis was performed using the matched t-test to compare values obtained before consumption and at the 6- and 12-week time points. Scores obtained by classification evaluation and the questionnaire were treated as nonparametric, and the Wilcoxon signed-rank test was used to compare findings within the groups. For comparison between groups, statistical analysis was performed using the unmatched t-test (two-sided test) to compare the BX-N and control groups at the 6- and 12-week time points. Scores obtained by classification evaluation and the questionnaire were treated as nonparametric,

and the Wilcoxon rank-sum test was used for intra-group comparisons. Statistical analysis was conducted using JMP (JMP 13, SAS Institute Japan Ltd., Minato-ku, Tokyo, Japan), and the significance level was set to less than 5% in the two-sided test.

Ethics

This study was conducted in compliance with the Declaration of Helsinki (revised at the 2013 WMA General Assembly in Fortaleza) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology Ministry of Health, Labor and Welfare). To ensure the rights and safety of the subjects, and reliability of the data, this study was conducted under the deliberation and approval of the Institutional Review Board for "Research Involving Human Subjects" of the Society for Glycative Stress Research (Approval number: GSR 2018 No. 005). This study was conducted after prior registration to the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) (registration number: UMIN000033706).

Results

Study completion status

The study included a total of 48 subjects with 12 subjects in each group; 1 subject from the BX-L group did not undergo the medical examination in week 12 due to personal reasons. The flow of the test subjects is shown in ***Fig. 1***. As stipulated in the study plan, when part of the data could not be obtained due to unavoidable reasons that were not related to the study, it was treated as missing data. Therefore, all 48 patients were included in the efficacy analysis (PPS analysis).

Primary endpoints

Results from the BX-N and control groups are shown in ***Table 3*** and ***Fig. 2***. The BX-N group showed a significant decrease in AST 12 weeks after consumption (22.0 ± 1.7 U/L) compared to before consumption (24.8 ± 1.5 U/L, $p = 0.015$), and a significant decrease in LDH 6 weeks (185.1 ± 9.3 U/L) and 12 weeks after consumption (173.8 ± 10.5 U/L) compared to before consumption (195.3 ± 10.9 U/L, $p = 0.007$, $p < 0.001$, respectively). The change in AST after 12 weeks

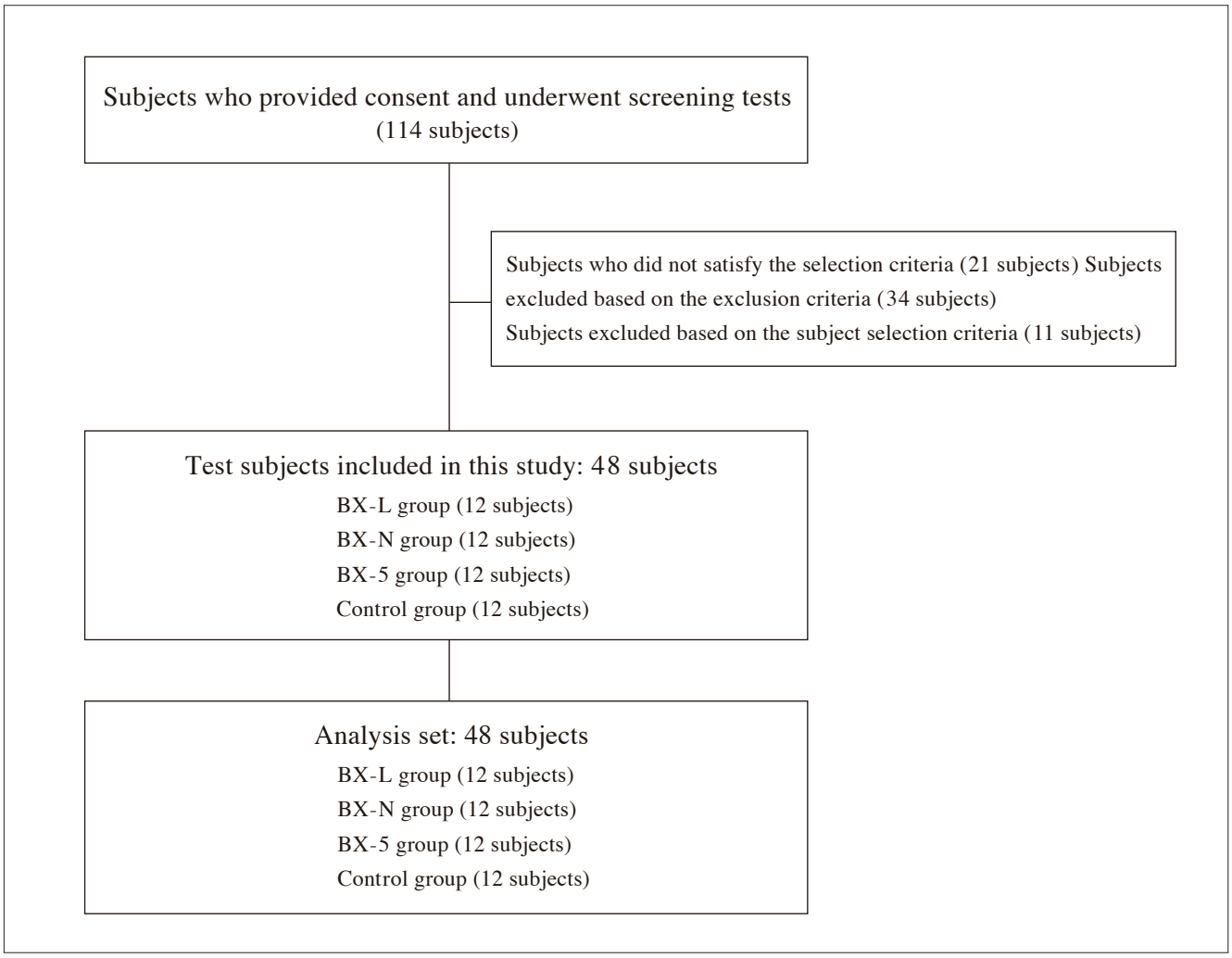

Fig. 1. **Flow chart of the clinical trial.**

Table 3. Evaluation items in primary endpoints

Item	Unit	Group	0 weeks	6 weeks	p value	12 weeks	p value
AST	U/L	BX-N	24.8 ± 1.5	23.8 ± 2.1	0.440	22.0 ± 1.7	0.015*
		Control	21.6 ± 1.3	23.3 ± 2.1	0.264	22.6 ± 2.1	0.575
ALT	U/L	BX-N	29.0 ± 3.8	28.1 ± 3.5	0.708	25.3 ± 3.4	0.190
		Control	21.5 ± 2.9	23.3 ± 3.9	0.334	20.9 ± 3.4	0.807
γ-GTP	U/L	BX-N	54.6 ± 13.3	60.4 ± 18.5	0.363	52.8 ± 16.5	0.713
		Control	58.8 ± 16.2	65.2 ± 16.7	0.193	59.4 ± 15.0	0.937
ALP	U/L	BX-N	205.0 ± 17.7	208.3 ± 18.8	0.548	202.0 ± 19.0	0.570
		Control	222.2 ± 11.5	211.2 ± 8.6	0.129	220.9 ± 9.3	0.874
LDH	U/L	BX-N	195.3 ± 10.9	185.1 ± 9.3	0.007**	173.8 ± 10.5	< 0.001**
		Control	195.3 ± 10.5	187.8 ± 8.7	0.291	172.6 ± 8.7	< 0.001**
ChE	U/L	BX-N	379.8 ± 11.1	385.1 ± 11.5	0.399	392.1 ± 11.3	0.086
		Control	386.0 ± 20.7	395.7 ± 18.3	0.129	399.5 ± 17.2	0.145

Data are expressed as mean ± SEM, paired t test, n = 12. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ChE, cholinesterase; SEM, standard error of the mean.

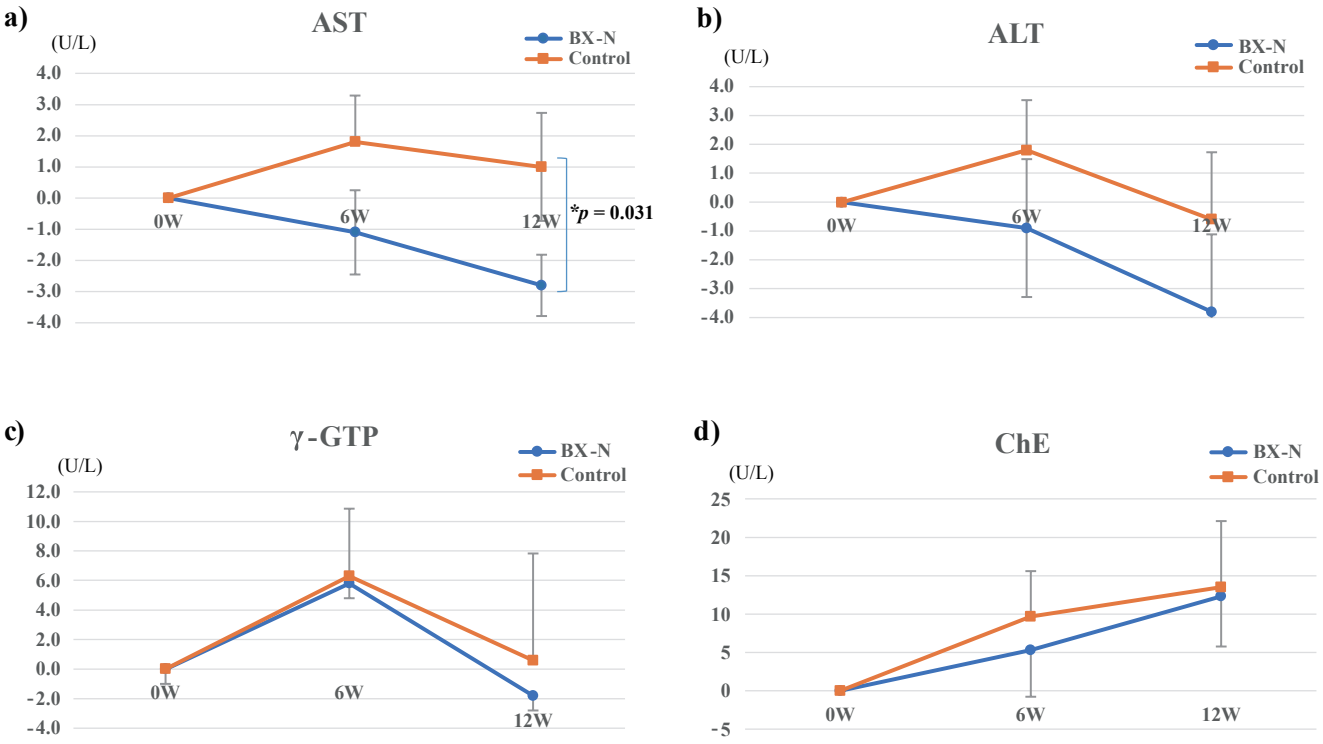

Fig. 2. **Changes in serum markers of liver function.**

a) AST, **b)** ALT, **c)** γ-GTP, **d)** ChE. Results are expressed as mean ± SEM, n = 12, Wilcoxon test. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ChE, cholinesterase; SEM, standard error of the mean.

was significantly greater in the BX-N group than in the control group ($p = 0.031$, ***Fig. 2a***). There were no significant changes in γ-GTP, ALP, or ChE. Additionally, there were no significant changes in the Anti-Aging QOL Common Questionnaire score.

Safety endpoints

Four adverse events were reported in 3 cases, the symptoms of which were all transient or caused by external factors. No causal relationship with the study or with the study diet was observed. The safety endpoints are shown in ***Table 4, 5*** and ***6***.

After 12 weeks, body fat in the BX-L group remained unchanged (+2.9%, p > 0.05), but was significantly increased in the BX-N group (+1.9%, p = 0.003), BX-5 group (+2.8%, p = 0.001) and control group (+1.8%, p = 0.024). However, no significant differences were observed between groups.

Systolic blood pressure was significantly increased in the BX-L group (+7.0%, p = 0.027), but remained unchanged in the BX-N group (+5.6%, p > 0.05), BX-5 group (+7.0%, p > 0.05) and control group (+1.7%, p > 0.05). However, no significant differences were observed between groups. Diastolic blood pressure was significantly increased in the BX-L group (+6.0%, p = 0.035) and BX-N group (+6.6%, p = 0.024), but remained unchanged in the BX-5 group (+2.9%, p > 0.05) and control group (+2.9%, p > 0.05). No significant differences were observed between groups.

Table 4. Anthropometry.

Item	Unit	Group	0 weeks	6 weeks	p value	12 weeks	p value
Height	cm	BX-L	162.98 ± 2.76	– ± –	–	– ± –	–
		BX-N	164.25 ± 2.38	– ± –	–	– ± –	–
		BX-5	165.03 ± 2.44	– ± –	–	– ± –	–
		Control	162.67 ± 2.43	– ± –	–	– ± –	–
Weight	kg	BX-L	69.8 ± 3.4	69.9 ± 3.4	0.796	69.7 ± 3.6	0.855
		BX-N	72.1 ± 3.2	72.6 ± 3.1	0.006**	72.1 ± 3.1	0.901
		BX-5	72.2 ± 2.2	71.9 ± 2.4	0.258	72.5 ± 2.3	0.211
		Control	70.6 ± 2.9	70.7 ± 3.0	0.679	70.7 ± 3.0	0.670
Body fat	%	BX-L	30.8 ± 1.7	30.9 ± 1.7	0.697	31.7 ± 1.8	0.332
		BX-N	32.1 ± 1.8	32.0 ± 1.8	0.982	32.7 ± 1.9	0.003**
		BX-5	31.9 ± 1.9	32.5 ± 1.9	0.006**	32.8 ± 1.9	0.001**
		Control	32.9 ± 1.7	33.3 ± 1.8	0.085	33.5 ± 1.7	0.024*
BMI	–	BX-L	26.1 ± 0.6	26.1 ± 0.6	0.914	26.2 ± 0.6	0.887
		BX-N	26.6 ± 0.6	26.8 ± 0.6	0.007**	26.6 ± 0.6	0.893
		BX-5	26.4 ± 0.3	26.3 ± 0.3	0.213	26.6 ± 0.3	0.267
		Control	26.6 ± 0.6	26.6 ± 0.6	0.857	26.6 ± 0.6	0.791
Blood pressure (systolic)	mmHg	BX-L	122.2 ± 3.7	129.0 ± 4.8	0.038*	130.8 ± 5.6	0.027*
		BX-N	127.7 ± 3.5	133.4 ± 4.3	0.153	134.8 ± 4.3	0.054
		BX-5	122.0 ± 3.3	128.9 ± 2.9	0.032*	130.6 ± 4.6	0.064
		Control	125.7 ± 4.2	131.6 ± 4.0	0.049*	127.8 ± 5.5	0.670
(diastolic)	mmHg	BX-L	80.3 ± 3.1	81.6 ± 3.0	0.524	85.1 ± 3.4	0.035*
		BX-N	82.4 ± 2.2	85.4 ± 3.1	0.209	87.8 ± 3.3	0.024*
		BX-5	79.4 ± 1.9	83.7 ± 2.6	0.034*	84.7 ± 4.0	0.168
		Control	80.6 ± 2.6	83.4 ± 3.0	0.035*	82.9 ± 3.1	0.208
Pulse	/min	BX-L	72.7 ± 1.7	72.5 ± 2.7	0.939	74.5 ± 3.0	0.600
		BX-N	72.6 ± 2.1	75.9 ± 2.2	0.167	74.7 ± 2.8	0.357
		BX-5	73.2 ± 1.9	72.6 ± 2.4	0.725	69.9 ± 2.6	0.054
		Control	73.8 ± 3.1	70.9 ± 2.2	0.068	75.0 ± 2.5	0.480

Data are expressed as mean ± SEM, paired t test, n = 12. BMI, body mass index; SEM, standard error of the mean.

Table 5. Blood chemistry.

Item	Unit	Group	0 weeks	12 weeks	p value
AST	U/L	BX-L	21.3 ± 1.9	19.0 ± 1.0	0.360
		BX-N	24.8 ± 1.5	22.0 ± 1.7	0.015*
		BX-5	21.7 ± 1.4	20.5 ± 0.9	0.284
		Control	21.6 ± 1.3	22.6 ± 2.1	0.575
ALT	U/L	BX-L	21.7 ± 3.1	17.5 ± 1.8	0.134
		BX-N	29.0 ± 3.8	25.3 ± 3.4	0.190
		BX-5	22.2 ± 3.0	20.2 ± 1.3	0.433
		Control	21.5 ± 2.9	20.9 ± 3.4	0.807
γ-GTP	U/L	BX-L	30.3 ± 4.7	25.9 ± 3.6	0.605
		BX-N	54.6 ± 13.3	52.8 ± 16.5	0.713
		BX-5	52.9 ± 18.5	51.2 ± 18.6	0.912
		Control	58.8 ± 16.2	59.4 ± 15.0	0.937
ALP	U/L	BX-L	204.5 ± 13.2	206.9 ± 13.4	0.971
		BX-N	205.0 ± 17.7	202.0 ± 19.0	0.570
		BX-5	187.6 ± 12.7	206.3 ± 16.2	0.134
		Control	222.2 ± 11.5	220.9 ± 9.3	0.874
LDH	U/L	BX-L	197.7 ± 10.2	179.5 ± 6.7	0.012*
		BX-N	195.3 ± 10.9	173.8 ± 10.5	< 0.001**
		BX-5	178.2 ± 5.6	167.3 ± 5.5	0.020*
		Control	195.3 ± 10.5	172.6 ± 8.7	< 0.001**
ChE	U/L	BX-L	358.8 ± 15.6	367.9 ± 19.1	0.210
		BX-N	379.8 ± 11.1	392.1 ± 11.3	0.086
		BX-5	378.6 ± 10.6	385.4 ± 15.8	0.577
		Control	386.0 ± 20.7	399.5 ± 17.2	0.145
TG	mg/dL	BX-L	136.7 ± 21.7	115.5 ± 22.3	0.361
		BX-N	145.1 ± 17.3	121.2 ± 8.4	0.047*
		BX-5	191.4 ± 33.3	158.2 ± 33.9	0.137
		Control	180.2 ± 20.0	155.1 ± 24.1	0.201
LDL-C	mg/dL	BX-L	134.2 ± 7.2	142.3 ± 9.2	0.436
		BX-N	149.7 ± 6.9	150.7 ± 7.5	0.755
		BX-5	134.8 ± 8.6	136.6 ± 8.6	0.843
		Control	147.3 ± 10.0	144.0 ± 7.6	0.645
TC	mg/dL	BX-L	223.1 ± 8.2	231.3 ± 9.3	0.351
		BX-N	238.4 ± 9.1	235.3 ± 9.4	0.458
		BX-5	218.3 ± 9.0	214.9 ± 9.7	0.740
		Control	234.3 ± 10.0	225.5 ± 8.6	0.227
HDL-C	mg/dL	BX-L	59.2 ± 6.4	68.3 ± 7.2	0.014*
		BX-N	60.5 ± 3.2	62.6 ± 3.3	0.086
		BX-5	51.1 ± 3.8	51.8 ± 3.3	0.749
		Control	53.3 ± 4.2	55.8 ± 4.5	0.061

Data are expressed as mean ± SEM, paired t test, n = 12. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ChE, cholinesterase; TG, triglycerides; LDL-C, low-density lipoprotein-C; TC, total cholesterol; HDL-C, high-density lipoprotein-C; SEM, standard error of the mean.

Table 6. Complete blood count and blood chemistry.

Item	Unit	Group	0 weeks	12 weeks	p value
WBC	/μL	BX-L	6500.0 ± 302.3	6563.6 ± 284.5	0.726
		BX-N	5350.0 ± 308.1	5325.0 ± 341.6	0.901
		BX-5	5675.0 ± 235.8	5850.0 ± 291.9	0.321
		Control	5933.3 ± 185.2	6266.7 ± 468.9	0.434
RBC	$\times 10^4$/μL	BX-L	477.5 ± 11.1	480.6 ± 13.7	0.415
		BX-N	472.0 ± 10.8	476.3 ± 10.3	0.316
		BX-5	463.6 ± 7.5	471.3 ± 8.3	0.300
		Control	479.2 ± 10.5	478.9 ± 11.3	0.972
Hb	g/dL	BX-L	14.22 ± 0.31	14.27 ± 0.36	0.629
		BX-N	14.37 ± 0.35	14.43 ± 0.33	0.626
		BX-5	14.18 ± 0.29	14.33 ± 0.34	0.555
		Control	14.50 ± 0.41	14.38 ± 0.46	0.609
Ht	%	BX-L	45.27 ± 0.79	44.21 ± 0.94	0.091
		BX-N	45.23 ± 0.96	44.20 ± 0.84	0.018*
		BX-5	44.78 ± 0.71	43.98 ± 0.70	0.278
		Control	45.76 ± 1.09	44.35 ± 1.05	0.059
MCV	fL	BX-L	95.0 ± 1.1	92.2 ± 1.1	< 0.001**
		BX-N	95.9 ± 0.8	92.8 ± 0.8	< 0.001**
		BX-5	96.8 ± 0.9	93.4 ± 1.0	< 0.001**
		Control	95.7 ± 1.2	92.8 ± 1.5	< 0.001**
MCH	pg	BX-L	29.81 ± 0.36	29.76 ± 0.39	0.434
		BX-N	30.44 ± 0.36	30.29 ± 0.33	0.296
		BX-5	30.59 ± 0.40	30.39 ± 0.42	0.082
		Control	30.25 ± 0.43	30.00 ± 0.59	0.213
MCHC	%	BX-L	31.39 ± 0.27	32.28 ± 0.21	< 0.001**
		BX-N	31.74 ± 0.22	32.60 ± 0.23	< 0.001**
		BX-5	31.65 ± 0.22	32.53 ± 0.32	< 0.001**
		Control	31.67 ± 0.23	32.33 ± 0.34	0.031*
TP	g/dL	BX-L	7.25 ± 0.10	7.25 ± 0.15	0.603
		BX-N	7.28 ± 0.08	7.33 ± 0.09	0.429
		BX-5	7.19 ± 0.14	7.21 ± 0.15	0.889
		Control	7.45 ± 0.12	7.40 ± 0.13	0.590
ALB	g/dL	BX-L	4.26 ± 0.07	4.37 ± 0.07	0.108
		BX-N	4.39 ± 0.04	4.48 ± 0.05	0.075
		BX-5	4.28 ± 0.07	4.34 ± 0.09	0.368
		Control	4.34 ± 0.08	4.41 ± 0.07	0.347
BUN	mg/dL	BX-L	12.02 ± 0.58	12.34 ± 0.46	0.396
		BX-N	14.00 ± 1.29	15.77 ± 1.06	0.019*
		BX-5	13.62 ± 1.02	13.51 ± 0.82	0.910
		Control	12.95 ± 0.56	13.73 ± 0.86	0.299
CRE	mg/dL	BX-L	0.743 ± 0.044	0.743 ± 0.040	0.061
		BX-N	0.811 ± 0.051	0.879 ± 0.050	0.011*
		BX-5	0.728 ± 0.040	0.778 ± 0.038	0.007**
		Control	0.768 ± 0.034	0.818 ± 0.041	0.025*
FPG	mg/dL	BX-L	81.0 ± 1.2	81.8 ± 1.7	0.578
		BX-N	84.5 ± 2.4	88.2 ± 2.7	0.033*
		BX-5	81.6 ± 1.8	81.5 ± 1.9	0.938
		Control	84.1 ± 2.0	83.8 ± 2.5	0.868
HbA1c [NGSP]	%	BX-L	5.67 ± 0.09	5.48 ± 0.08	< 0.001**
		BX-N	5.61 ± 0.06	5.48 ± 0.07	0.018*
		BX-5	5.57 ± 0.06	5.43 ± 0.07	0.003**
		Control	5.63 ± 0.06	5.48 ± 0.05	< 0.001**

Data are expressed as mean ± SEM, paired t test, n = 12. WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; TP, total protein; ALB, albumin; BUN, urea nitrogen; CRE, creatinine; FPG, fasting plasma glucose; HbA1c hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program; SEM, standard error of the mean.

Discussion

Given that animal experiments have confirmed that BX dry powder improves liver function, the first aim of this clinical study was to determine whether it exhibits a similar effect on improving liver function in humans. The second aim was to evaluate the safety of a dose of BX dry powder that is 5 times the standard dose. The method used was a stratified randomized, double-blind, parallel-group comparison study for subjects with high ChE, AST and ALT levels.

Improvement of liver function following a standard BX dose

While there no significant changes in biochemical indices (AST, ALT and γ-GTP) of the liver in the control group, BX-L, BX-N, and BX-5 groups, which received BX, showed improvements. In particular, the BX-N group showed a significant decrease in AST levels after 12 weeks with the other groups. The BX-L and BX-N groups showed dose-dependent changes in AST, ALT, and γ-GTP levels compared to the control group. The effect of BX between the BX-5 group and BX-N group was only similar on γ-GTP, while its effect on AST and ALT was weaker in the BX-5 than the BX-N group. No adverse effects due to consumption of BX were observed. These results suggest that consumption of food containing a standard dose of BX improved liver function.

ChE is an indicator of fatty liver and was used to screen the subjects participating in this study. Higher ChE levels indicate progression of fatty liver. Blood biochemistry results showed that ChE levels tended to decrease in the BX-L and BX-5 groups compared to the control group. However, no significant difference or dose-dependent change was observed between groups. Anti-obesity studies using obese rats have shown a significant improvement in body weight gain and fatty liver in obese rats fed food mixed with BX dry powder [4, 5]. Similar to these animal experiments, the findings in the present study suggest that administration of a high dose of BX (BX-5 group) may also improve fatty liver in humans.

The main components of the test food are BX powder, which contains the active ingredient 4-(2-formy-5-hydroxymethyl-pyrrole-1-yl)-butanoic acid, and a new antioxidant with a structure in which the carboxylic acid group is converted to a carboxylic acid amide [1, 2]. A significant feature of BX powder is its potent antioxidant activity for scavenging hydroxy radicals [1, 2]. Further, 4-(2-formy-5-hydroxymethyl-pyrrole-1-yl)-butanoic acid has been shown to have hepatoprotective effects [8]. The antioxidant action of BX may be one of the mechanisms underlying its hepatoprotective effect. A previous clinical study conducted by the authors of the present study showed that astaxanthin significantly improved AST [9]. The results of that study suggested that the antioxidant effect suppresses the production of lipid peroxide in the blood and liver, thereby reducing the load on hepatocytes and facilitating the release of enzymes such as AST and ALT.

Safety evaluation

BX is an edible mushroom with a long history of consumption as a food. There are no reports of adverse effects as a result of consumption of BX, indicating it to be a very safe food.

Anti-obesity studies conducted using obese rats have shown that body weight gain was significantly suppressed in those fed food mixed with BX dry powder [4, 5]. In contrast, we did not observe weight suppression in any group. Although the body fat percentage was significantly higher in the control, BX-L and BX-N groups, there were no significant differences between the BX groups compared to the control group. Studies have reported the presence of a seasonal variation in body fat percentage [10, 11]. A longitudinal study of healthy elderly people living in Ishikawa Prefecture (22 females, 17 males, average age 70.7 ± 3.2 years) conducted over 7 years from the summer of 2004 to the winter of 2011 showed that body fat percentage was higher in winter than in summer [12]. Further, the results of a study that compared female students from a number of countries showed that there were seasonal changes in body fat percentage in Japan, Thailand and Poland [13, 14]. Slight changes in body fat percentage were observed in the present study, ranging from +1.9% to +2.9% in the 3 groups that received BX and +1.8% in the control group, which are within the range of seasonal variation. We therefore concluded that the effect of BX on body fat was safe.

The functional component 4-(2-formy-5-hydroxymethylpyrrole-1-yl)-butanoic acid contained in BX dry powder has antioxidant effects. Antioxidants relieve vascular wall tension and induce high-pressure effects generally as vascular smooth muscle expands due to exposure to nitric oxide (NO) derived from endothelial cells and contracts when exposed to free radicals and reactive oxygen species (ROS) [15-17]. Previous clinical studies have shown a significant decrease in blood pressure with intake of astaxanthin [9] and anthocyanidin derived from cassis [18]. Therefore, we expected consumption of BX to reduce blood pressure. Because BX does not contain components that can affect autonomic nerves, BX is unlikely to affect blood pressure through sympathetic or parasympathetic nerve activity. However, blood pressure measurements showed that the systolic blood pressure was significantly increased in the BX-L group, while the diastolic blood pressure was significantly increased in the BX-L and BX-N groups. We speculate that the increase in blood pressure was due to the effect of climate. Generally, cardiovascular events and mortality rates increase when the outdoor temperature decreases in winter [19-21]. Blood pressure shows a seasonal variation, decreasing in summer and increasing in winter, and is affected by various factors such as age, sex, and obesity [22]. The measured temperatures during this study were 29.6°C (10:00 AM), maximum 34.1°C, minimum 23.4°C in week 0 (September 3, before study initiation); 19.5°C (10:00 AM), maximum 25.3°C, minimum 12.6°C in week 6 (October 26); and 14.2°C (10:00 AM), maximum 15.7°C, minimum 12.0°C in week 12 (December 7). Compared to weeks 0 and 6, the temperature measured in week 12 was colder, and blood pressure was likely to have been higher.

Temperature fluctuations also affect morning blood pressure surge (MBPS), a proposed indicator of blood pressure circadian fluctuations related to the onset and prognosis of cardiovascular diseases because onset often occurs in the morning [23, 24]. There are 2 types of MBPS: pre-waking MBPS [25], which describes the increase in blood pressure 2 hours before and after waking, and sleep-through MBPS [26], which describes an increase in blood pressure from the lowest blood pressure at night for 2 hours into the early morning. A study by Saeki et al. divided 146 healthy subjects (average age 32.0 years) into the cold exposure group, who were kept at a room temperature of 14°C (76 people), and the control group, who were kept at a room temperature of 24°C (70 people). Subjects were free to adjust the number of clothes worn from 2 hours before sleeping to 2 hours after waking up, and blood pressure was measured at 15-minute intervals to calculate and compare the MBPS between the 2 groups [27]. The sleep-through MBPS was 7.2 mmHg (95% confidence interval: 3.9 to 10.5) and pre-waking MBPS was 5.2 mmHg (95% confidence interval: 2.1 to 8.2), even though the number of clothes worn by the cold exposure group was significantly greater than the control group. In this study, the average systolic blood pressure increased by 7.1 to 8.4 mmHg, similar to the MBPS, and is in line with the hypothesis that the increased blood pressure was caused by cold exposure.

We speculate that seasonal changes such as a decrease in outdoor temperature are significant factors governing the increase in blood pressure observed during the present study. Our findings showed that the blood pressure fluctuation was within the physiological range, high-dose BX-5 had no effect on blood pressure, and there were no significant differences between the BX groups and the control group. Therefore, seasonal changes such as the decrease in outdoor temperature were major factors for the observed increase in blood pressure, and we concluded that the effects of BX on blood pressure were safe.

Possible effect on resistance to cold

The degree of blood pressure increase differed by group. After 12 weeks, systolic blood pressure was significantly increased in the BX-L group, and diastolic blood pressure was significantly increased in the BX-L and BX-N groups, while no significant differences were observed in the control or BX-5 group. We hypothesize that BX dry powder may have changed the subjects' resistance to cold environments. As mentioned above, blood pressure is lower in summer when the outdoor temperature is high, and higher in winter when the outdoor temperature is low. The difference between the outdoor temperature and room temperature has a significant effect on blood pressure, although blood pressure can increase at low room temperatures even when the outdoor temperature is the same as that indoors [22]. In the present study, the room temperature was not controlled and was left to the discretion of each subject. Therefore, the subjects were free set a higher temperature when they felt cold or to leave the room temperature unchanged if they were comfortable. We speculate that BX consumption increased the subjects' adaptability to low-temperature environments (cold resistance), making it unnecessary to increase the room temperature.

One of the mechanisms governing cold resistance is an increase in the amount of brown adipose tissue (BAT) [28-30]. BAT is a type of adipose tissue with high thermogenic ability and contributes to maintaining the internal body temperature at low external temperatures. The intensity of BAT activity is inversely correlated with the degree of obesity in adults [31]. Recent studies have revealed the presence of 2 types of thermogenic adipose tissue: BAT and beige adipose tissue, which play an important role in controlling systemic energy metabolism. When a homoiothermic animal is exposed to cold, brown/beige adipocytes induce rapid thermogenesis via the sympathetic nerves. Additionally, the expression of genes involved in fat burning and thermogenesis is rapidly induced, enhancing the thermogenic ability. A sustained cold environment further stimulates the differentiation of white adipose tissue (WAT) to beige adipose tissue [32, 33]. This phenomenon is also called "beiging" of WAT and is a mechanism used by the body to adapt to chronic cold environments. Thermogenesis in BAT is induced by dependence on uncoupling protein-1 (UCP1). Obesity, type 2 diabetes, fatty liver, and age are factors that decrease UCP1 [34, 35]. When these factors improve, the thermogenesis ability of BAT is restored. The increased thermogenesis of BAT is reproduced by capsaicin and tea catechins that stimulate bile acids, β3 adrenergic receptor agonists and the temperature receptor TRP [36,37]. Subjects in the present study who showed improvements in obesity and fatty liver may have exhibited a recovery of the thermogenesis ability of BAT, and thereby improved adaptability to the cold environment. In contrast, beige adipocytes use a novel thermogenesis pathway independent of UCP1. Induction of beige adipocytes is expected to lead to suppression of obesity and improved systemic glucose and lipid metabolism. PRDM16, a transcription coregulator, and EHMT1, a histone modifier, are closely involved in the development and differentiation of beige adipocytes. While casein kinase CK2 suppresses the differentiation of beige adipocytes in mice, administration of a CK2 inhibitor induces beiging of WAT, and increases UCP1 expression and energy consumption [38]. The effects of the functional ingredients contained in BX on CK2 are currently unknown and require further investigation.

Conclusion

BX dry powder was used to evaluate the efficacy of a standard dose and the safety of a high dose of BX. The BX standard dose (BX-N) group showed improved liver function (AST and LDH), with AST levels being significantly improved compared to the control group. This suggests that the functional ingredients contained in BX improved fatty liver through antioxidation and inhibition of lipid peroxidation. Further, no adverse events were observed, even when a high dose (5 times the usual dose) was consumed for 12 weeks, confirming the safety of BX. BX is therefore expected to be a useful component of functional foods aimed at improving fatty liver and liver function. Detailed mechanisms underlying its improvement of liver function require further investigation.

Conflict of Interest Statement

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