Doctoral Thesis

# Studies on Physiological Functions and Clinical Application of the Fermented Barley Extract P

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Studies on Physiological Functions and Clinical Application of the Fermented Barley Extract P

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Hideki Hokazono, Toshiro Omori, Hiramitsu Suzuki and Kazuhisa Ono

Food Science and Technology Research, **15** (6), 599-604 (2009).

(2) Effects of Single and Combined Administration of Fermented Barley Extract and γ-Aminobutyric Acid on the Development of Atopic Dermatitis in NC/Nga Mice

Hideki Hokazono, Toshiro Omori and Kazuhisa Ono

Bioscience, Biotechnology, and Biochemistry, **74** (1), 135-139 (2010).

(3) Anti-hyperuricemic Effect of Fermented Barley Extract is Associated with Increased Urinary Uric Acid Excretion

Hideki Hokazono, Toshiro Omori and Kazuhisa Ono

Food Science and Technology Research, **16** (4), 295-304 (2010).

(4) Effects of a Fermented Barley Extract on Subjects with Slightly High Serum Uric Acid or Mild Hyperuricemia

Hideki Hokazono, Toshiro Omori, Tetsuro Yamamoto, Ieo Akaoka and Kazuhisa Ono

Bioscience, Biotechnology, and Biochemistry, **74** (4), 828-834 (2010).

## 3. Reference thesis

(1) Effects of Several By-Products of Alcoholic Beverage on the Fatty Liver in Rats Fed Orotic Acid.

Satoshi Mochizuki, Akiko Miyamoto, Miwako Hagiwara, Noriko Yasumatsu, Rie Wakabayashi, Hideki Hokazono, Naoki Takeshima and Toshiro Omori

Research Bulletin of the Faculty of Education and Welfare Science, Oita University, **27** (2), 197-206 (2005).

(2) Effects of Barley *Shochu* Lees on D-Galactosamine-Induced Hepatic Injury in Rats.

Satoshi Mochizuki, Akiko Miyamoto, Saiko Oga, Chiharu Kai, Kouhei Oshima, Toshikazu Tanaka, Hideki Hokazono, Naoki Takeshima and Toshiro Omori

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(3) Production of γ-Aminobutyric Acid and S-Adenosylmethionine in Barley-*Shochu* Making

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Journal of the Brewing Society of Japan, **100** (12), 877-881 (2005).

# Main thesis

# Contents

# Abbreviations

| | |
|---|---|
| AD | atopic dermatitis |
| Alb | albumin |
| APCs | antigen-presenting cells |
| BMI | body-mass index |
| BP | blood pressure |
| BUN | blood urea nitrogen |
| BW | body weight |
| $Cl_{ur}$ | uric acid clearance |
| CMC | carboxymethyl cellulose |
| Cr | creatinine |
| FBE | fermented barley extract |
| FBEP | fermented barley extract P |
| FBF | fermented barley fiber |
| FBS | fasting blood sugar |
| GABA | γ-aminobutyric acid |
| GPx | glutathione peroxidase |
| GSH | glutathione |
| Hb | hemoglobin |
| HDL-C | HDL-cholesterol |
| HMC | hematocrit |
| hOAT | human organic anion transporter |
| Ht | height |
| IFN | interferon |
| Ig | immunoglobulin |
| IL | interleukin |
| LDL-C | LDL-cholesterol |
| MDA | malondialdehyde |
| NA | noradrenalin |
| PB | pearled barley |
| PiCl | picryl chloride |
| PSL | prednisolone |
| RBC | red blood cell |
| RH | relative humidity |
| SDB | *shochu* distillery by-products |
| s-IgA | secretory IgA |
| SOD | superoxide dismutase |
| TBARS | thiobarbituric acid-reactive substances |
| Tbil | total bilirubin |
| Tcho | total cholesterol |
| TG | triglyceride |
| TP | total protein |
| UA | uric acid |
| URAT | human urate transporter |
| WBC | white blood cell |
| XOD | xanthine oxidase |

# Chapter 1
# Introduction

Single-distilled *shochu* (*honkaku-shochu* in Japanese) is a traditional Japanese spirit mainly produced and consumed in the southern islands of Kyushu and Okinawa, Japan. However, the consumption and production of *shochu* continue to expand nationally after the boom of the 1980s. Japan Sake Brewers Association announced that the taxable shipment volume of *honkaku-shochu* for the 2008 fiscal year was 506,161 kL, a 5-fold increase over 30 years. Fig. 1-1 shows transition of taxable shipment volume of *honkaku-shochu*. Recent increases in *shochu* production have resulted in an enormous output of *shochu* distillery by-products (SDB), exceeding approximately 800,000 tons per year. Treatment of SDB presents challenges, as it is a highly concentrated organic waste liquid with ≥ 90% moisture content and is not easily filtered. In the past, SDB was neutralized and dumped in the sea as an industrial waste. However, as public awareness of global environmental issues increase, Japan ratified the London Convention (Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter 1972) for the prevention of marine pollution in 1980. Since the regulations of the convention were strengthened in 1996, Japan revised the Law Relating to the Prevention of Marine Pollution and Disaster in 2004 and enacted it in 2007, in principle banning the dumping of wastes from ships. Furthermore, the Food Recycling Law (Law Concerning the Promotion of Recycling Food Cyclical

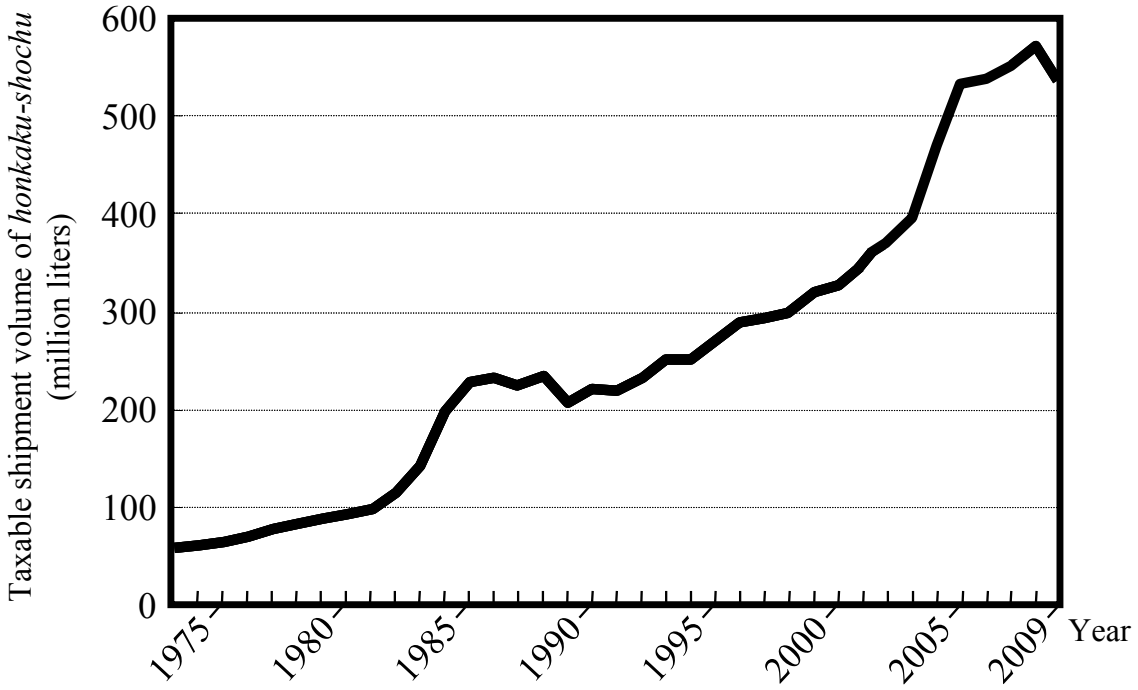


**Fig. 1-1. Transition of taxable shipment volume of *honkaku-shochu*.**

Resources) requires SDB to be not simply treated as an industrial waste, but as an unused resource where its effective utilization would result in minimization of its environmental load. In an effort to comply with this regulation, many *shochu* producers attempted to treat all SDB on land. Treatment facilities exclusively for SDB were introduced in their plants to convert this by-product into feed and fertilizer. However, this approach has several drawbacks, including high costs of initial investment and operation. Conversely, SDB is a precious resource rich in useful components derived from raw materials, microorganisms, or fermentation processes.[1)] That is, in addition to recycle SDB as a raw material for feed and fertilizer, its development as a food material for humans based on investigations on its nutritional and physiological functions could lead to further uses or added value. This perspective has encouraged research into treatment of this by-product, by applying novel processing and utilization technologies and elucidating its physiological functions.

To meet a variety of applications, our company passed barley-SDB through a 1-mm sieve and filtered the permeated liquid through a 0.2-μm ceramic filter to obtain fermented barley extract (FBE), a clear filtrate. The filtrate was separated into non-adhering and adhering fractions with a synthetic adsorbent. The non-adhering fraction has been used as a material in beverages and seasonings, or as a medium for microbiological culture.[2)] The fermented barley extract P (FBEP) used in this study, which is an FBE fraction adhering to the synthetic adsorbent, contains peptides, oligosaccharides, soluble dietary fiber, and polyphenols.

FBE was reported to suppress orotic acid-induced fatty liver and D-galactosamine-induced hepatic disorder.[3-5] These hepatic disorders are peculiar to rats and were reported to involve oxidative stress, inflammation, and disruption of nucleic acid metabolism during disease onset and development.[6,7]

In this study, we elucidated the physiological functions of FBE and FBEP through a pre-clinical test using animal models of inflammation and disorders of nucleic acid metabolism and through a clinical application study in humans. The purpose was to effectively utilize SDB, an unused/under-utilized resource, as a functional food material that is beneficial to humans and to advanced technologies.

Chapter 2 examines the effects of FBE and FBEP on *in vivo* antioxidative activities in mice. FBE is the liquid remaining after the distillation of mash, which is produced by enzymatic degradation of pearled barley using a *koji* mold and alcohol fermentation using yeast. FBE contains a variety of useful components derived from barley and microorganisms that are decomposed or formed during saccharification and fermentation processes. Several reports indicate that one of the physiological functions of barley is improved lipid metabolism in animals and humans.[8-12] In addition to the aforementioned suppression of hepatic disorders, barley-SDB was reported to have *in vivo* physiological functions, such as the alleviation of ovalbumin-sensitized rhinitis in mice[13] and reduction of blood sugar levels in rats with streptozotocin-induced diabetes.[14] Although there are several reports using experimental animals to investigate the effects of barley and SDB on high cholesterol, high-fat food, or

disease model systems, few reports have evaluated the long-term effects of basic feed compositions. Hence, chapter 2 discusses the results of a 3-month mixed feed administration conducted by feeding adult mice FBE, FBEP, and pearled barley (a raw material for *shochu*). An evaluation was conducted on the effects of the mixed feed on plasma, hepatic lipid and antioxidant parameters.

Chapter 3 examines the effects on inflammatory dermatosis. Attention to living environment and mental stress, in addition to inflammation control, pruritus control, and skin care, is important for the treatment of atopic dermatitis (AD), in which pruritus and eczema are the main lesions. In recent years, plant polyphenols have been noted as an antiallergic food ingredient.[15,16] There are epidemiological reports that indicate low asthma morbidity in people who took high levels of flavonoids.[17] Thus, FBEP, in which barley polyphenols are extracted and concentrated, is expected to suppress allergies caused by antioxidative and anti-inflammatory activities. γ-Aminobutyric acid (GABA), which is noted for decreasing blood pressure[18,19] and anti-stress activity,[20] was reported to lead to the activation of cell functions, such as the production of hyaluronic acid and glutathione, an *in vivo* antioxidant.[21] Therefore, GABA is expected to suppress AD through anti-inflammatory activity and improved skin barrier function. Thus, chapter 3 discusses how FBEP and GABA administered singly or in combination influence the development of AD-like skin lesions in NC/Nga mice by continuous application of the hapten picryl chloride (PiCl).

Chapter 4 examines the effects of FBEP on nucleic acid

metabolism. Abnormalities in purine and pyrimidine metabolism are believed to play a role in fat accumulation in the liver of rats fed orotic acid.[7] Conversely, allopurinol, which suppresses uric acid (UA) production, was reported to suppress fat accumulation in the liver of rats fed orotic acid.[22] Additionally, it has been reported that levels of human urate transporter 1 (URAT 1) are greatly reduced by high levels (1 mM) of orotic acid,[23] and that URAT 1 is responsible for transporting orotic acid across cell membranes. Furthermore, this transport is effectively blocked by benzbromarone, a UA excretion-promoting drug.[24] Thus, FBEP may affect nucleic acid metabolism. Therefore, chapter 4 presents an examination of the effects of FBEP administration on three strains of rats with anomalies in nucleic acid metabolism (hyperuricemia models).

Chapter 5 describes a clinical test of FBEP effects on UA metabolism in humans. The Japanese Society of Gout and Nucleic Acid Metabolism defines hyperuricemia as a condition characterized by a serum UA level > 7.0 mg/dL, irrespective of gender and age. Hyperuricemia, an underlying cause of gout, is present in about 20% of male adults in Japan.[25] Additionally, hyperuricemia is an independent predictive factor for metabolic syndrome, which frequently leads to complications related to life style, such as high blood pressure, glucose tolerance disorder, and obesity.[26,27] Early detection and treatment are imperative in the control of gout and hyperuricemia. For the treatment of gout and hyperuricemia, it is desirable to begin controlling UA levels when slightly elevated serum UA levels appear, which does not meet the applicable criterion of

pharmacological treatment. This situation has highlighted the need for food that could control UA levels. In an exploratory open study, FBEP reduced serum UA in subjects with slightly elevated levels.[28] Hence, to statistically confirm the effect of FBEP on decreasing serum UA levels, taking into consideration the effects of environmental factors and the placebo effect, and having studied the safety of FBEP, a double-blind parallel-group comparison study was conducted with Japanese men and women with slightly elevated serum UA levels (6.5-7.5 mg/dL).

# Chapter 2
# Effects of Fermented Barley Extract on Antioxidant Status in Mice

## 2.1 Introduction

Genuine *shochu* is a traditional Japanese spirit made from barley, rice, sweet potato, sugar cane or buckwheat. Japanese *shochu* production is characterized by the use of *koji* and by its method of distillation. Whereas malt is used in the fermentation of whiskey and beer, *koji* is used in *shochu* fermentation. *Koji* is peculiar to Asia and is produced by culturing fungi of the *Aspergillus* family (suited to temperate and humid climates) using cereal substrates such as rice or barley. Recent increases in *shochu* production have resulted in an enormous output of SDB. In the past, most of the SDB was dumped at sea, as industrial waste. However, the 1996 protocol of the London Convention stimulated research to develop treatment methods in lieu of dumping, and to investigate any useful characteristics of SDB as a non-utilized resource. Subsequently, many methods have been developed to effectively utilize SDB. For example, there have been reports on the use of SDB as dried feed or fertilizer,[29] a growth promoter for broilers,[30] a seasoning,[31] and in anticancer food.[32] SDB contains an abundance of cereal- and microbial-derived nutrients, and its composition varies depending on the raw material, raw material formula and distillation method. It has previously been reported on the effects of barley-SDB in promoting the growth of enteric bacteria,[33] preventing fatty liver induction by orotic acid,[3]

and preventing liver injury caused by D-galactosamine.[5] Since barley-SDB has been found to provide health benefits (as described above), barley-SDB was renamed "Fermented barley (FB)" to avoid the negative waste-product connotation. However, investigation into the influence of FB fraction intake on animal and human health is necessary. Two experiments using mice were conducted in an effort to elucidate the effects of oral administration of the extract fraction from FB on the body.

## 2.2 Materials and Methods

*Materials* Pearled barley (PB; barley pearled to approximately 65% of its initial weight), purchased from Ishibashikohgyo (Fukuoka, Japan), was ground into flour and used in the experiment. FB, provided by Sanwa Shurui (Oita, Japan), was prepared using PB as the raw material. To produce steamed barley, PB was allowed to absorb water until a 40% w/w water content was achieved, steamed for 40 min, and subsequently cooled to 40°C. The barley-*koji* was produced by inoculating the steamed barley with white *koji* mold (*Aspergillus kawachii*) (1 kg/ton of barley), which was maintained at 38°C and 95% relative humidity (RH) for 24 h with a subsequent incubation at 32°C and 92% RH for 20 h. The seed mash was prepared with 3.6 kL water, 30 L of *shochu*-yeast (*Saccharomyces cerevisiae*) preculture ($3 \times 10^8$ cfu/mL) and 3 tons of barley-*koji*. The seed mash was fermented for 5 days at 25°C. The main mash was prepared by adding 7 tons of steamed barley and 11.4 kL of water to the seed mash. The main mash was fermented for 11 days at 25°C. After fermentation,

the main mash was subjected to single batch distillation to obtain 10 kL of unrefined barley-*shochu* and 15 kL of FB. FB was filtered with a stainless-steel mesh net (1 mm). The resulting residual substance, named fermented barley fiber (FBF), was dried with heated air and milled. The extracted liquid was then filtered using a ceramic filter (porosity 0.2 μm) to remove solid residues, such as plant cell walls and microbial cells. Subsequently, the aqueous solution, named fermented barley extract (FBE), was freeze-dried and mixed with 50% w/w water-soluble dextrin (Pinedex 100™; Matsutani Chemical Industry, Hyogo, Japan) as an excipient. A 10-liter volume of FBE before concentration and drying was subjected to an Amberlite FPX66™ (Rohm and Haas, Philadelphia, USA) column (80 × 10 cm I.D.) equilibrated with distilled water. After washing with distilled water, the trapped material was eluted with 75% ethanol. The eluted fraction was freeze-dried. As a result, approximately 100 g of freeze-dried sample, named FBEP, was obtained from 10 kg of PB. The recovery rates of FB, FBE and FBEP from PB were 20%, 10% and 1% on a dry weight basis, respectively. The nutritional components of the samples were analyzed and are shown in Table 2-1.

*Animals* Male Crlj:CD-1 (ICR) mice (5 weeks old) were obtained from Charles River Japan (Kanagawa, Japan). All animals were switched from a laboratory diet, MF (Oriental Yeast, Tokyo), to the experimental diets at 5 months of age. Forty-eight (Exp. 1) or thirty-two (Exp. 2) mice were randomly divided into six or four groups of eight animals each, and housed in suspended stainless-steel cages with wire mesh bottoms. The animal room was kept at 24 ±

**Table 2-1. Nutritional components of samples (g/100 g)**

| Components | PB | FBF | FBE | FBEP |
|---|---|---|---|---|
| Moisture | 15.8 | 6.0 | 5.1 | 6.2 |
| Crude protein | 6.0 | 19.6 | 18.9 | 39.2 |
| Crude fat | 1.3 | 13.3 | 0.0 | 0.3 |
| Ash | 0.7 | 1.9 | 2.6 | 3.7 |
| Total dietary fiber | 7.2 | 47.4 | 0.9 | 8.8 |
| Soluble fiber | (4.9) | (1.7) | (0.9) | (8.8) |
| Insoluble fiber | (2.3) | (45.7) | (0.0) | (0.0) |

PB, pearled barley flour; FBF, fermented barley fiber; FBE, fermented barley extract; FBEP, fermented barley extract P.

0.5°C with the RH at 65 ± 5%. Room lighting consisted of 12-hour periods of light and dark. The mice were given free access to the diets and water. All the mice were fed the experimental diets for 3 months. Food consumption (g/day) was observed and body weights were measured once a week. All the mice were maintained according to the guidelines for experimental animals of the National Food Research Institute, Japan.

*Diet* Each diet contained 5% lipid, 20% protein and 48.8% carbohydrate sources, in addition to the following components: granulated sugar, 15%; cellulose powder, 5%; salt mixture, 4%; vitamin mixture, 2%; and L-methionine, 0.2%. The salt and vitamin mixtures were purchased from Oriental Yeast (Tokyo). In these diets, lard and casein were used as lipid and protein sources, respectively. Furthermore, dextrin and corn starch were used as carbohydrate sources. In Exp. 1, the diets containing 0% or 10% of samples (PB, FBF or FBE) were used. In Exp. 2, the diets containing 0% of sample, 10% of FBE or 5% of FBEP were used. Amounts of lipid, protein and carbohydrate contained in experimental samples were deducted from the amount of lard, casein and cornstarch of the control diet in the preparation of experimental diets, respectively.

*Preparation of plasma and erythrocyte lysate samples* At the end of the feeding trials, all the mice were fasted for 20 hours before being anesthetized with diethyl ether. Blood was then collected from the inferior vena cava with a heparinized syringe and transferred to ice-cold tubes. Plasma and erythrocytes were separated by centrifugation at 900×g for 20 min at 4°C. In Exp. 2, erythrocytes

were subsequently washed once with 10 volumes of cold 0.9% NaCl solution by centrifugation at 900×g for 20 min at 4°C. The washed centrifuged erythrocytes were lysed by 4 volumes of cold deionized water, mixed and left at 4°C for 15 min. Plasma and erythrocyte lysate samples were stored at –30°C until required for analysis.

*Preparation of liver homogenates* After blood collection, the whole liver of each mouse was removed and homogenized with 1/15 mol/L phosphate buffered saline (pH 7.4) using a Teflon-glass homogenizer. Tissue homogenates were stored at –30°C until required for analysis.

*Analytical procedures* Total cholesterol, triacylglycerol and phospholipid concentrations in plasma samples and liver homogenates were determined by the enzymatic methods of Allain *et al.*,[34] Spayd *et al.*,[35] and Takayama *et al.*,[36] respectively. Plasma free fatty acid concentrations (in Exp. 1) were measured by the enzymatic method of Shimizu *et al.*[37] The thiobarbituric acid-reactive substances (TBARS) concentrations in plasma were determined by the method of Yagi,[38] and those in tissues were determined by the method of Ohkawa *et al.*,[39] with a minor modification. The TBARS concentrations are presented as nmol malondialdehyde (MDA), using tetraethoxypropane as an external standard. In Exp. 2, erythrocyte glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were determined using commercially available kits (BIOXYTECH GPx-340™ and SOD-525™, respectively). Liver glutathione (GSH) concentration was determined using a BIOXYTECH GSH-420™ colorimetric assay kit. All of these kits

were purchased from OXIS International (Portland, USA).

*Statistical Analyses* All results were expressed as means ± SD. The statistical significance of differences between dietary groups was determined by one-way analysis of variance using the STATISTICA statistical program package (StatSoft, Okla., USA). When the F test was significant, comparisons between the dietary groups were performed using Duncan's multiple range test at $p<0.05$.

## 2.3 Results

*Exp. 1.* Food consumption was 4.5 ± 0.1 g/day, and the differences between the dietary groups were not significant. The final body weights were as follows: control group, 50.0 ± 4.5 g; PB group, 48.8 ± 5.0 g; FBF group, 49.4 ± 4.7 g; FBE group, 48.3 ± 4.8 g. There were no significant differences in body weight between groups.

The effects of experimental diets on plasma lipid components and TBARS are shown in Table 2-2. There were no significant differences in total cholesterol concentration among the groups. Triacylglycerol concentration was significantly lower in the PB group than in the control group. Phospholipid concentration tended to be higher in the FBF group and lower in the PB group. Free fatty acid concentrations were significantly lower in PB flour and FB groups, and especially so in the FBE group, as compared to the control group. Only the FBE group was significantly lower in TBARS concentration than the control group.

The effects on liver lipids and TBARS are shown in Table 2-3. Total cholesterol concentration was significantly higher in the PB

**Table 2-2. Effect of PB, FBF and FBE on plasma lipids in mice (Exp. 1)**

| | Dietary group | | | |
|---|---|---|---|---|
| | Control | PB | FBF | FBE |
| Total cholesterol (mg/dL) | 158.7±25.8 | 139.0±23.0 | 162.5±20.9 | 134.8±34.8 |
| Triacylglycerol (mg/dL) | 107.1±34.3$^{b}$ | 63.1±8.5$^{a}$ | 107.3±19.2$^{b}$ | 98.6±47.0$^{ab}$ |
| Phospholipids (mg/dL) | 225.2±31.6$^{ab}$ | 184.9±24.8$^{a}$ | 238.7±28.3$^{b}$ | 203.7±37.1$^{ab}$ |
| Free fatty acid (μEq/L) | 917.2±104.1$^{c}$ | 647.0±70.7$^{ab}$ | 636.0±61.9$^{ab}$ | 564.5±103.0$^{a}$ |
| TBARS (nmol/mL) | 37.0±9.4$^{bc}$ | 28.9±5.1$^{abc}$ | 37.5±6.2$^{c}$ | 26.4±4.8$^{a}$ |

PB, pearled barley flour; FBF, fermented barley fiber; FBE, fermented barley extract; TBARS, thiobarbituric acid-reactive substances. Each value is expressed as the mean ± SD (n = 8). Values for each dietary group with different small letters in the same lipid fractions are significantly different at $p<0.05$ by Duncan's multiple range test.

**Table 2-3. Effect of PB, FBF and FBE on liver lipids in mice (Exp. 1)**

| | Dietary group | | | |
|---|---|---|---|---|
| | Control | PB | FBF | FBE |
| Total cholesterol (mg/g tissue) | 4.6±0.6[a] | 6.8±1.6[b] | 6.8±0.6[b] | 5.8±1.2[ab] |
| Triacylglycerol (mg/g tissue) | 46.3±16.5[ab] | 38.1±11.7[ab] | 56.9±8.8[b] | 61.1±24.2[b] |
| Phospholipids (mg/g tissue) | 16.8±0.9[a] | 17.4±0.9[ab] | 19.3±1.3[b] | 17.7±2.5[ab] |
| TBARS (nmol/g tissue) | 70.8±4.3[b] | 70.2±4.1[b] | 68.3±3.9[b] | 62.9±3.0[a] |

PB, pearled barley flour; FBF, fermented barley fiber; FBE, fermented barley extract; TBARS, thiobarbituric acid-reactive substances. Each value is expressed as the mean ± SD (n = 8). Values for each dietary group with different small letters in the same lipid fractions are significantly different at $p<0.05$ by Duncan's multiple range test.

and FBF groups than in the control group. Phospholipid concentration tended to be higher in the FBF group. TBARS concentration was significantly lower in the FBE group than in the control, PB, and FBF groups.

*Exp. 2.* Food consumption was 4.4 ± 0.2 g/day, and the differences between the dietary groups were not significant. The final body weights were as follows: control group, 52.0 ± 3.1 g; FBE group, 51.1 ± 4.4 g; FBEP group, 50.2 ± 3.2 g. There were no significant differences in body weight between groups.

The plasma lipid concentration for each dietary group is presented in Table 2-4. No significant differences were found in total cholesterol, triacylglycerol or phospholipid concentrations among the groups. TBARS concentration was significantly lower in the FBE and FBEP groups than in the control group.

The erythrocyte GPx and SOD activities are shown in Table 2-5. Erythrocyte GPx activity was significantly higher in the FBEP group than in all the other groups. Erythrocyte SOD activity did not differ significantly among the groups.

The liver TBARS and GSH concentrations are presented in Table 2-6. TBARS concentration was significantly lower in the FBE and FBEP groups than in the control group. Hepatic GSH was significantly higher in the FBEP group than in the control group.

## 2.4 Discussion

There have been several reports demonstrating the beneficial physiological effects of barley on lipid metabolism in animals and

**Table 2-4. Effect of FBE and FBEP on plasma lipids in mice (Exp. 2)**

| | Dietary group | | |
|---|---|---|---|
| | Control | FBE | FBEP |
| Total cholesterol (mg/dL) | 171.8±38.9 | 174.8±23.7 | 157.2±28.1 |
| Triacylglycerol (mg/dL) | 107.9±23.9 | 91.4±16.0 | 94.5±17.6 |
| Phospholipids (mg/dL) | 261.7±47.5 | 249.8±30.4 | 226.7±38.6 |
| TBARS (nmol/mL) | 37.4±14.8[b] | 22.0±6.0[a] | 23.9±7.5[a] |

FBE, fermented barley extract; FBEP, fermented barley extract P; TBARS, thiobarbituric acid-reactive substances. Each value is expressed as the mean ± SD (n = 8). Values for each dietary group with different small letters in the same lipid fractions are significantly different at $p<0.05$ by Duncan's multiple range test.

**Table 2-5. Erythrocyte GPx and SOD activities of mice fed experimental diets for 3 months (Exp. 2)**

| | Dietary group | | |
|---|---|---|---|
| | Control | FBE | FBEP |
| GPx (U/mL RBC) | 40.5 ± 6.8[a] | 40.3 ± 3.6[a] | 60.4 ± 4.8[b] |
| SOD (U/mL RBC) | 560.6 ± 31.8 | 543.8 ± 31.2 | 538.6 ± 27.6 |

GPx, glutathione peroxidase; SOD, superoxide dismutase; RBC, red blood cell; FBE, fermented barley extract; FBEP, fermented barley extract P. Each value is expressed as the mean ± SD (n = 8). Values for each dietary group with different small letters in the same column are significantly different at $p<0.05$ by Duncan's multiple range test.

**Table 2-6. Liver TBARS and GSH concentrations of mice fed experimental diets for 3 months (Exp. 2)**

| | Dietary group | | |
|---|---|---|---|
| | Control | FBE | FBEP |
| TBARS (nmol/g tissue) | 43.1 ± 2.7[b] | 37.4 ± 2.1[a] | 35.9 ± 3.6[a] |
| GSH (mmol/g tissue) | 4.8 ± 0.3[a] | 5.3 ± 0.4[ab] | 5.9 ± 0.4[b] |

TBARS, thiobarbituric acid-reactive substances; GSH, glutathione; FBE, fermented barley extract; FBEP, fermented barley extract P. Each value is expressed as the mean ± SD (n = 8). Values for each dietary group with different small letters in the same column are significantly different at $p<0.05$ by Duncan's multiple range test.

humans.[8-12] With respect to the animal experiments, many studies concerned themselves with high cholesterol and high-fat food, but few reports evaluated the long-term effect of barley on lipid metabolism in animals given a diet of ordinary nutritional composition. When the present experimental data is analyzed from the perspective of PB, which is the raw material of FBF and FBE, the results are as follows. Plasma triacylglycerol concentration was significantly lower in the PB group than in the control group, whereas the FBE group was not different. With respect to liver lipid levels, the total cholesterol value was significantly higher in the PB group than in the control group, but did not differ significantly between FBE and control groups. Thus, it was suggested that FBE had little effect on lipid metabolism in mice fed a low fat diet. We thought that the reason FBE had no significant effect on lipid metabolism was that soluble dietary fiber (particularly beta-glucan) from barley had been degraded by fungal enzymes during the fermentation process. In addition, the safety of 3 months continuous administration of FBE and FBEP was confirmed in mice, as no abnormal variations in the organ weight and necropsy findings were observed.

TBARS concentrations in the plasma and liver were significantly lower in the FBE group than in the control group. Antioxidants, such as polyphenols, are believed to be involved in this effect. Some polyphenols have been identified in barley, and are reported to be proanthocyanidin oligomers.[40,41] These polyphenols have reportedly shown strong antioxidant activity, and firmly trap hydrophilic radicals in aqueous solution systems.[42,43] However,

components other than polyphenols may have been involved, as the FBE and FBEP prepared in this study were unrefined. Furthermore, because barley, a raw material of *shochu*, was not found to decrease the amount of systemic TBARS, the FBE components accentuating antioxidant properties in the body may have been due to an increase in antioxidant activity or the absorption of nutritional components produced, by fungi or yeast, during fermentation.[44,45] That is, it may be inferred that the fungi form an antioxidant, enzymatically decompose polyphenol glycosides, and produce a highly absorbable aglycon or an antioxidant peptide. *In vitro* anti-oxidant activity of the absorbed fraction of ethanol extract of PB on the hydrophobic resin was the same as for FBEP (data not shown). Further study on identifying the substances in FBEP that enhance antioxidant activity *in vivo* is required.

Results of numerous experiments have shown that dietary components rich in polyphenols and exhibiting high antioxidant capacity can influence biomarkers of antioxidants in rats, for example increased GPx and SOD activities.[46-48] Cellular antioxidant enzymes such as GPx and SOD have a central role in the control of reactive oxygen species. In mammalian cells, GSH and GPx constitute the principal antioxidant defense system.[49,50] In the present experiment, investigation of the *in vivo* effects on antioxidant index showed that erythrocyte GPx activity was significantly higher in the FBEP group than in the other groups. Moreover, hepatic GSH was significantly higher in the FBEP group than in the control group. GPx is an antioxidant enzyme that renders hydrogen peroxide or

peroxidized lipid nontoxic by a two-electron reduction in the presence of GSH. GPx works together with SOD and catalase to play a role in the body's defense system against oxidative stress. GSH is known to be a very important component involved in toxin metabolism and antioxidant systems involved in protecting liver function.[51-53] The increase in the amount of liver GSH of animals fed FBEP is judged to be a piece of the evidence that supports the protective effect of FB on liver functions.[3-5] It was thought that consumption of GSH was suppressed as a result of increasing the activity of GPx by FBEP. Moreover, it is necessary to investigate the influence of FBEP on glutathione S-transferase (not evaluated in this study) and to clarify a detailed mechanism of the *in vivo* antioxidative activity of FBEP.

These findings show that FBEP has the effect of significantly reducing oxidative stress in the body. We consider it necessary to identify the components involved in these functions and, in the future, confirm their effects on humans.

## 2.5 Summary

Effects of dietary supplementation with Fermented barley extract (FBE) on lipid metabolism and antioxidant status in mice were studied. In Exp. 1, male 5-month-old mice were fed either a control diet or an experimental diet containing 10% barley, fermented barley fiber (striated part of barley grain) or FBE for 3 months. In Exp. 2, male 5-month-old mice were fed either a control diet or an experimental diet containing FBE or FBEP (adsorbed fraction of FBE

on the hydrophobic resin) for 3 months. The thiobarbituric acid-reactive substances (TBARS) concentrations in both plasma and liver were significantly lower in the FBE and FBEP groups than the control group. Erythrocyte glutathione peroxidase (GPx) activity was significantly higher in the FBEP group than in the other groups. Liver glutathione (GSH) content was significantly higher in the FBEP group than in the control group. These results suggest that FBEP, prepared from the water-soluble fraction of barley-*shochu* distillery by-products, significantly reduces oxidative stress in the body.

# Chapter 3
# Effects of Single and Combined Administration of Fermented Barley Extract and γ-Aminobutyric Acid on the Development of Atopic Dermatitis in NC/Nga Mice.

## 3.1 Introduction

Atopic dermatitis (AD) is an illness characterized by pruriginous eczema as its main morbid process, in which aggravation and abatement are repeated, and most AD patients present an atopic disposition.[54] Allergic reactions to antigens, namely immunologic disorders and disorders of skin barrier function such as atopic dry skin, are important factors in the development of AD. Therefore, based on dualistic thought, the control of inflammation, a reduction in pruritus, and skin care are important in the treatment of AD. Moreover, since environment factors and mental stress are complicating issues in the development and aggravation of dermatitis, it is important to pay attention to such conditions. While foods contain allergens, they also contain ingredients that provide preventive and palliative effects on allergic reactions. In recent years, anti-allergic foods utilizing the functionality of such food ingredients have been attracting attention, and lactic acid bacteria and plant polyphenols have been the main focus of reports.[15,16,55,56]

In chapter 2, oral administration of FBEP in mice resulted in reduced oxidative stress by controlling glutathione-redox. On the other hand, Iguchi *et al*. reported that administration of FBE to mice with ovalbumin-sensitized rhinitis reduced the increase in frequency

of sneezing and nose-scratching.[57,58] Furthermore, the development of AD-like skin lesions in NC/Nga mice was reduced by alterations in the cytokines involved in chronic inflammation, such as interferon (IFN)-γ and interleukin (IL)-17. In addition, epidemiological studies have indicated that a low incidence of asthma was observed in a population with a high intake of flavonoids.[17] Hence it is expected that the anti-oxidative and anti-inflammatory actions of FBEP (polyphenol fraction of FBE) will result in allergy-reducing effects.

On the other hand, several expression aberrations of neuro-transmitters have been reported in the skin of AD patients.[59-61] It was reported that noradrenalin (NA), a major neurotransmitter involved in the control of the vasoconstrictive motion of human skin, was most enriched in the skin of AD patients and might be one of the pathological, aggravating factors of AD by increasing inflammatory cytokine production.[62] Kimura *et al.*[63] and Hayakawa *et al.*[64] confirmed that GABA showed a hypotensive effect in spontaneously hypertensive rats by suppressing the release of NA in the peripheral sympathetic nerves. In addition, it was found that GAD67, a GABA-producing enzyme, was present in the dermal fibroblasts, and that GABA was involved in the activation of cellular function, for example in promoting the production of hyaluronic acid and the antioxidant glutathione.[21] Thus GABA might exhibit anti-inflammatory and AD-mitigating effects by improving skin barrier function.

This chapter examined the influence of single and combined administration of FBEP and GABA on the progress of AD-like skin

lesions in NC/Nga mice by continuous application of the hapten PiCl.

## 3.2 Materials and Methods

*Materials* The preparation of FBE is described in Chapter 2. Preparation of FBEP was as follows: A 10-liter volume of FBE, before concentration and drying, was subjected to an Amberlite FPX66™ (Rohm and Haas, Philadelphia, USA) column (80 × 10 cm I.D.) equilibrated with water. After washing with water, the trapped material was eluted with 1 wt-% NaOH. The eluted fraction was subjected to an Amberlite IRC76™ hydrogen form (Rohm and Haas) column. The pass-through fraction was freeze-dried. As a result, approximately 100 g of freeze-dried sample (FBEP) was obtained from 10 kg of PB. The composition of the FBEP was as follows: 41.8% carbohydrate, 39.2% protein, 8.8% dietary fiber, 0.3% fat, 3.7% ash, 6.2% water, and 8.5% polyphenol.

GABA (> 90% purity) was produced by natural fermentation using the lactic acid bacterium *Enterococcus* sp. FC 301 (Lacto-Fermented Barley GABA, Barley Fermentation Technologies, Oita, Japan).

*Animals* Female NC/Nga mice (7 weeks old) were obtained from Charles River Japan (Atsugi, Japan). The mice were housed in polycarbonate cages. The animal room was kept at 23 ± 2°C and the relative humidity at 30-80%. Room lighting consisted of a 12-hour light/dark cycle. The mice were given free access to diet (CRF-1, Oriental Yeast, Tokyo) and water. They were subjected to experimentation after a 1-week period of quarantine and domestication. The animal

experiments were performed in accordance with the "Standards Relating to the Care and Management of Experimental Animals (Notification No. 6, March 27, 1980, of the Prime Minister's Office, Japan, revised May 28, 2002)" and "The Japan Act on the Welfare and Management of Animals (up to the revisions of Act No. 50 of 2006)." In order to make the average body weight and standard deviations almost equal among the groups, the mice were divided into 10 groups: a normal control group (untreated), a control group (PiCl-treated), two FBE groups (the diets contained FBE at 2% or 10%), two FBEP groups (the diets contained FBEP at 0.5% or 2.5%), two GABA groups (the diets contained GABA at 0.1% or 0.25%), an FBEP-GABA group (the diet contained 2.5% FBEP and 0.25% GABA), and a prednisolone (PSL) group (3 mg/kg/day was administered orally using a stomach sonde) (n = 8 each group). The CRF-1 diet was given to the normal control, control, and PSL groups. The test sample groups mixed each test sample to achieve the aforesaid additive rate in the CRF-1 diet. Food consumption (g/day) was observed and body weights were measured once a week. The normal control group received no treatment, and the other seven groups were treated with PiCl, as described below.

*AD model mice* The abdominal and dorsal skin of the NC/Nga mice was shaved, and the shaved skin and sole of the foot were sensitized with 150 μL of 5% PiCl in an ethanol and acetone mixture (4:1). On day 4 after sensitization, the left auricle was challenged with 30 μL of 0.8% PiCl dissolved in olive oil. Challenge with 0.8% PiCl was repeated once a week for 10 weeks. Auricular

thickness was measured every week with a digital thickness gauge (Peacock G-7C type, Ozaki MFG, Tokyo).

*Measurement of serum IgE* Blood was collected by exsanguination from the heart at necropsy. Serum samples were obtained by centrifugation and stored at –80°C until use. The total serum IgE level was determined by ELISA (Shibayagi, Gunma, Japan).

*Measurement of cytokine production* All the mice were sacrificed and the splenocytes were collected. The splenocytes were washed with RPMI-1640 medium with 10% fetal bovine serum, and then suspended at a concentration of $1.2 \times 10^7$ cells/mL. The cells were cultured in a 24-well culture plate with anti-CD3 mAb (2 μg/mL) at 37°C for 48 h in a 5% $CO_2$ incubator. The culture supernatant was collected by centrifugation and stored at –80°C until assay. The concentrations of IFN-γ and IL-4 were determined with an ELISA kit (eBioscience, San Diego, CA).

*Statistical analysis* Data were expressed as means ± SE. Student's *t*-test was used to detect significant differences between the normal control group and the control group. One-way ANOVA with Bonferroni/Dunn's *post hoc* test was used to test for significant differences between groups. The *p* values less than 0.05 were considered statistically significant.

## 3.3 Results

No significant differences between the average food intakes of the groups were observed during the study period. Table 3-1 shows

**Table 3-1. Changes in body weight of NC/Nga mice in the various groups after initial sensitization**

| Group | (g) | Weeks after sensitization | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Normal control | Mean | 18.1 | 19.4 | 20.2 | 20.6 | 21.3 | 22.0 | 22.3 | 22.7 | 23.1 | 23.3 | 23.7 |
| | SE | 0.4 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 | 0.4 |
| Control | Mean | 18.1 | 19.4 | 19.5 | 19.7 | 20.5 | 20.7 | 21.1 | 21.4 | 21.6 | 21.9 | 22.5 |
| | SE | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 |
| FBE (2%) | Mean | 18.1 | 19.9 | 20.8 | 21.2 | 22.1 | 22.5 | 22.5 | 23.1 | 23.2 | 23.9 | 24.1 |
| | SE | 0.4 | 0.5 | 0.4 | 0.4 | 0.5 | 0.6 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| FBE (10%) | Mean | 18.1 | 18.3 | 20.0 | 20.6 | 21.5 | 21.7 | 21.7 | 22.0 | 22.1 | 22.5 | 23.0 |
| | SE | 0.6 | 0.8 | 0.5 | 0.7 | 0.6 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.6 |
| FBEP (0.5%) | Mean | 18.7 | 18.9 | 20.1 | 20.6 | 21.4 | 21.7 | 22.1 | 22.2 | 22.7 | 23.0 | 23.3 |
| | SE | 0.5 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.4 | 0.5 | 0.5 | 0.7 |
| FBEP (2.5%) | Mean | 18.8 | 19.3 | 20.6 | 21.2 | 21.8 | 21.8 | 22.4 | 22.5 | 22.6 | 22.9 | 23.4 |
| | SE | 0.4 | 0.7 | 0.7 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.7 |
| GABA (0.1%) | Mean | 18.3 | 18.9 | 19.9 | 20.5 | 21.2 | 21.5 | 21.8 | 22.0 | 22.4 | 22.7 | 23.2 |
| | SE | 0.3 | 0.7 | 0.4 | 0.5 | 0.4 | 0.5 | 0.5 | 0.5 | 0.5 | 0.6 | 0.6 |
| GABA (0.25%) | Mean | 18.5 | 18.5 | 19.6 | 20.2 | 20.8 | 21.3 | 21.8 | 22.0 | 22.4 | 22.6 | 22.7 |
| | SE | 0.5 | 0.8 | 0.4 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.4 |
| FBEP-GABA | Mean | 18.3 | 18.8 | 20.2 | 20.6 | 21.2 | 21.4 | 21.7 | 21.9 | 22.1 | 22.4 | 22.8 |
| | SE | 0.3 | 0.6 | 0.3 | 0.3 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 |
| PSL | Mean | 18.4 | 18.5 | 19.1 | 19.7 | 20.0 | 19.9 | 20.3 | 20.4 | 20.3 | 20.8 | 21.0 |
| | SE | 0.3 | 0.5 | 0.4 | 0.4 | 0.5 | 0.4* | 0.4* | 0.4** | 0.4** | 0.5** | 0.5** |

FBE, fermented barley extract; FBEP, fermented barley extract P; GABA, γ-aminobutyric acid; PSL, prednisolone. Body weight was measured once a week.
*$p<0.05$ and **$p<0.01$, significant difference as compared to the control group.

the changes in body weight of the groups during the study period. The body weight of the PSL group was significantly lower than the PiCl-treated control group 5 weeks after the start of sensitization until the end of the study period. For the other groups, no significant differences were observed among the groups.

*Ear thickness* Table 3-2 shows the changes in auricular thickness for the groups during the study period. No change in the thickness of the auricle of the normal control group was observed, whereas the auricle of the PiCl-treated control group showed a significant increase in thickness as compared with the normal control group 1 week after the onset of sensitization. Although the auricular thickness of each group administered the test substances increased at each time of repeated induction in the same manner as in the PiCl-treated control group, the increase in thickness was reduced as compared to the PiCl-treated control group 1-3 weeks after the onset of sensitization to the end of the study period. The dose-dependent, thickness-reducing effect of each test substance (FBE, FBEP, and GABA) was confirmed, but no additive/synergistic effects of combined administration of FBEP and GABA were observed. The thickness of the auricular area of the PSL group was further reduced as compared to each group administered the test substances, and was significantly thinner than the PiCl-treated control group throughout the study period.

*Serum IgE* Fig. 3-1 shows the serum IgE levels of the various groups. The PiCl-treated control group showed significantly higher serum IgE levels than the normal control group under sensitization and 10 weeks of induction treatment. Compared to the PiCl-treated control

**Table 3-2. Effects of consecutive administration of test substances on ear thickness induced by PiCl application in NC/Nga mice**

| Group | ($\times 10^3$mm) | Weeks after sensitization | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Normal control | Mean | 165.4 | 165.1 | 164.9 | 163.7 | 163.3 | 162.0 | 161.6 | 163.0 | 163.9 | 164.3 | 165.3 |
| | SE | 1.0 | 1.0 | 1.6 | 0.8 | 0.7 | 0.6 | 0.5 | 0.5 | 0.3 | 0.4 | 0.3 |
| Control | Mean | 164.8 | 169.6 | 177.4 | 194.4 | 203.9 | 217.8 | 233.1 | 249.9 | 268.5 | 281.9 | 291.9 |
| | SE | 2.1 | 1.6$^{\#}$ | 2.6$^{\#\#}$ | 2.4$^{\#\#\#}$ | 2.0$^{\#\#\#}$ | 4.8$^{\#\#\#}$ | 6.3$^{\#\#\#}$ | 8.9$^{\#\#\#}$ | 10.5$^{\#\#\#}$ | 11.0$^{\#\#\#}$ | 12.9$^{\#\#\#}$ |
| FBE (2%) | Mean | 169.8 | 167.5 | 172.5 | 182.1 | 191.8 | 206.1 | 212.1 | 221.1 | 238.4 | 246.9 | 257.0 |
| | SE | 1.4 | 0.8 | 2.8 | 1.9$^{***}$ | 2.6$^{***}$ | 4.2$^{*}$ | 6.0$^{*}$ | 5.2$^{*}$ | 6.4$^{*}$ | 5.8$^{*}$ | 6.9$^{*}$ |
| FBE (10%) | Mean | 166.4 | 166.4 | 174.7 | 184.1 | 190.6 | 199.4 | 206.9 | 212.0 | 227.0 | 240.7 | 249.1 |
| | SE | 1.0 | 0.6 | 2.6$^{*}$ | 1.4$^{**}$ | 1.0$^{***}$ | 2.3$^{**}$ | 4.8$^{**}$ | 6.1$^{***}$ | 7.8$^{**}$ | 8.2$^{**}$ | 9.1$^{**}$ |
| FBEP (0.5%) | Mean | 163.0 | 164.1 | 172.1 | 177.1 | 183.9 | 194.0 | 204.3 | 209.1 | 230.4 | 242.5 | 249.4 |
| | SE | 0.9 | 0.8 | 2.1 | 1.1$^{***}$ | 1.4$^{***}$ | 3.4$^{***}$ | 6.0$^{***}$ | 4.8$^{***}$ | 5.7$^{**}$ | 6.2$^{**}$ | 6.1$^{**}$ |
| FBEP (2.5%) | Mean | 163.1 | 164.0 | 173.3 | 177.9 | 185.4 | 192.6 | 197.1 | 205.0 | 217.0 | 231.3 | 234.0 |
| | SE | 0.8 | 1.0 | 3.3 | 1.8$^{***}$ | 2.4$^{***}$ | 4.3$^{***}$ | 6.4$^{***}$ | 8.7$^{***}$ | 10.0$^{***}$ | 9.6$^{***}$ | 12.3$^{***}$ |
| GABA (0.1%) | Mean | 163.9 | 164.9 | 166.6 | 175.3 | 180.6 | 184.8 | 190.8 | 207.0 | 226.3 | 237.8 | 245.6 |
| | SE | 1.1 | 0.6$^{**}$ | 1.8$^{**}$ | 1.9$^{***}$ | 1.8$^{***}$ | 2.4$^{***}$ | 3.6$^{***}$ | 5.5$^{***}$ | 5.6$^{***}$ | 6.1$^{**}$ | 5.3$^{**}$ |
| GABA (0.25%) | Mean | 164.8 | 164.3 | 165.9 | 175.3 | 179.4 | 185.6 | 191.1 | 203.4 | 221.0 | 231.3 | 238.3 |
| | SE | 0.8 | 0.9$^{**}$ | 0.7$^{**}$ | 1.8$^{***}$ | 1.6$^{***}$ | 3.1$^{***}$ | 4.1$^{***}$ | 7.0$^{***}$ | 7.2$^{***}$ | 10.8$^{***}$ | 11.1$^{***}$ |
| FBEP-GABA | Mean | 164.6 | 165.3 | 166.9 | 174.8 | 183.6 | 189.0 | 191.8 | 203.4 | 220.5 | 232.5 | 243.6 |
| | SE | 1.0 | 0.9$^{*}$ | 1.5$^{**}$ | 1.5$^{***}$ | 1.0$^{***}$ | 2.3$^{***}$ | 3.8$^{***}$ | 5.3$^{***}$ | 6.6$^{***}$ | 7.1$^{***}$ | 5.7$^{**}$ |
| PSL | Mean | 166.8 | 164.9 | 162.5 | 165.1 | 170.6 | 169.0 | 167.6 | 171.3 | 179.8 | 191.8 | 195.4 |
| | SE | 0.6 | 0.9$^{**}$ | 2.0$^{***}$ | 2.1$^{***}$ | 2.2$^{***}$ | 1.9$^{***}$ | 2.0$^{***}$ | 3.1$^{***}$ | 4.3$^{***}$ | 7.5$^{***}$ | 8.1$^{***}$ |

PiCl, picryl chloride; FBE, fermented barley extract; FBEP, fermented barley extract P; GABA, γ-aminobutyric acid; PSL, prednisolone. Body weight was measured once a week.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$, significant difference as compared to the normal control group.

$^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, significant difference as compared to the control group.

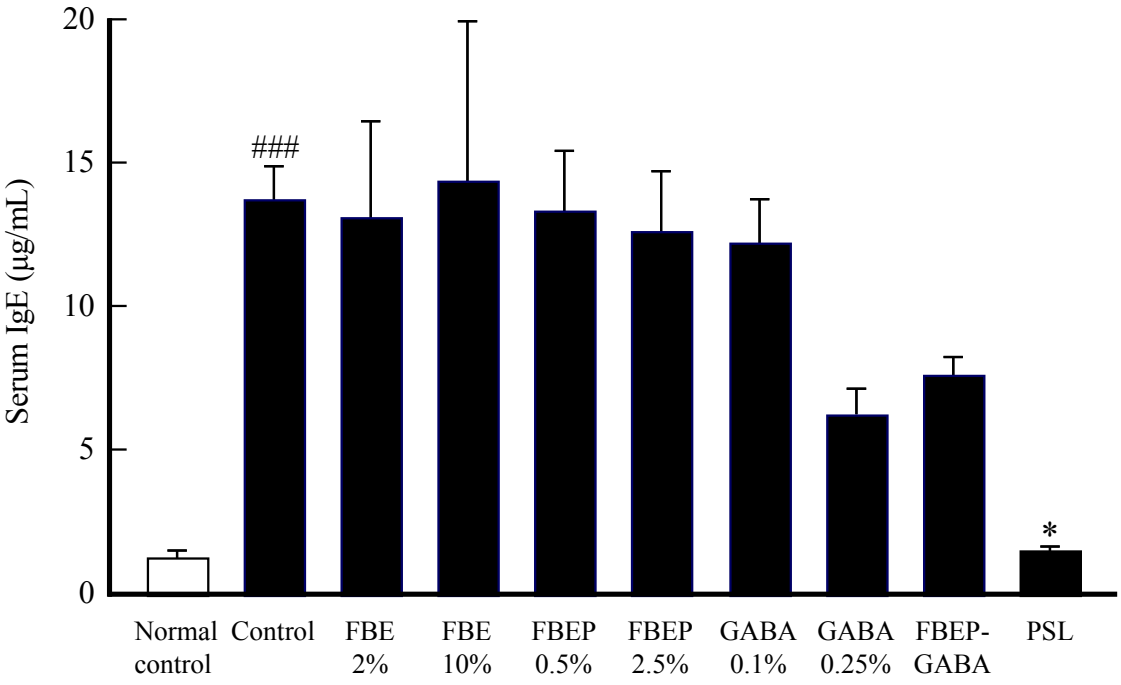


**Fig. 3-1. Effects of test substances on serum IgE levels in NC/Nga mice.**

Sera were collected at 10 weeks after the initial challenge with PiCl. Total IgE levels were determined by ELISA. Each bar represents the mean ± SE for eight mice. $^{###}p<0.001$, significant difference as compared to the normal control group. $^{*}p<0.05$, significant difference as compared to the control group.

group, the serum IgE of the PSL group showed significantly lower levels. The other groups administered either of the test substances showed no significant differences, but the serum IgE levels of the GABA (high dose) and FBEP-GABA groups tended to be low.

*IL-4* Fig. 3-2A shows splenocyte IL-4 production by the various groups. The PiCl-treated control group showed significantly higher levels of splenocyte IL-4 than the normal control group, whereas the PSL group showed significantly lower levels than the PiCl-treated control group. The GABA-administered groups had a dose-dependent tendency to show lower splenocyte IL-4 levels, while the high-dose groups showed a significant reduction in IL-4 levels as compared to the PiCl-treated control group. The FBEP-GABA group showed significantly lower IL-4 levels than the PiCl-treated control group.

*IFN-γ* Fig. 3-2B shows the splenocyte IFN-γ production of each group. The PiCl-treated control group showed significantly lower IFN-γ levels than the normal control group. The single administration group for each test substance (FBE, FBEP, and GABA) showed almost no difference in INF-γ level compared to the PiCl-treated control group, but the IFN-γ levels in the FBEP-GABA group was significantly higher than in the PiCl-treated control group, but its level was found to be equal to those of the normal control and PSL groups.

## 3.4 Discussion

The morbid state of AD is thought to be the result of allergic reactions of IgE-mediated type I (immediate and delayed type) and type IV (delayed type),[65] and an animal model in which individual

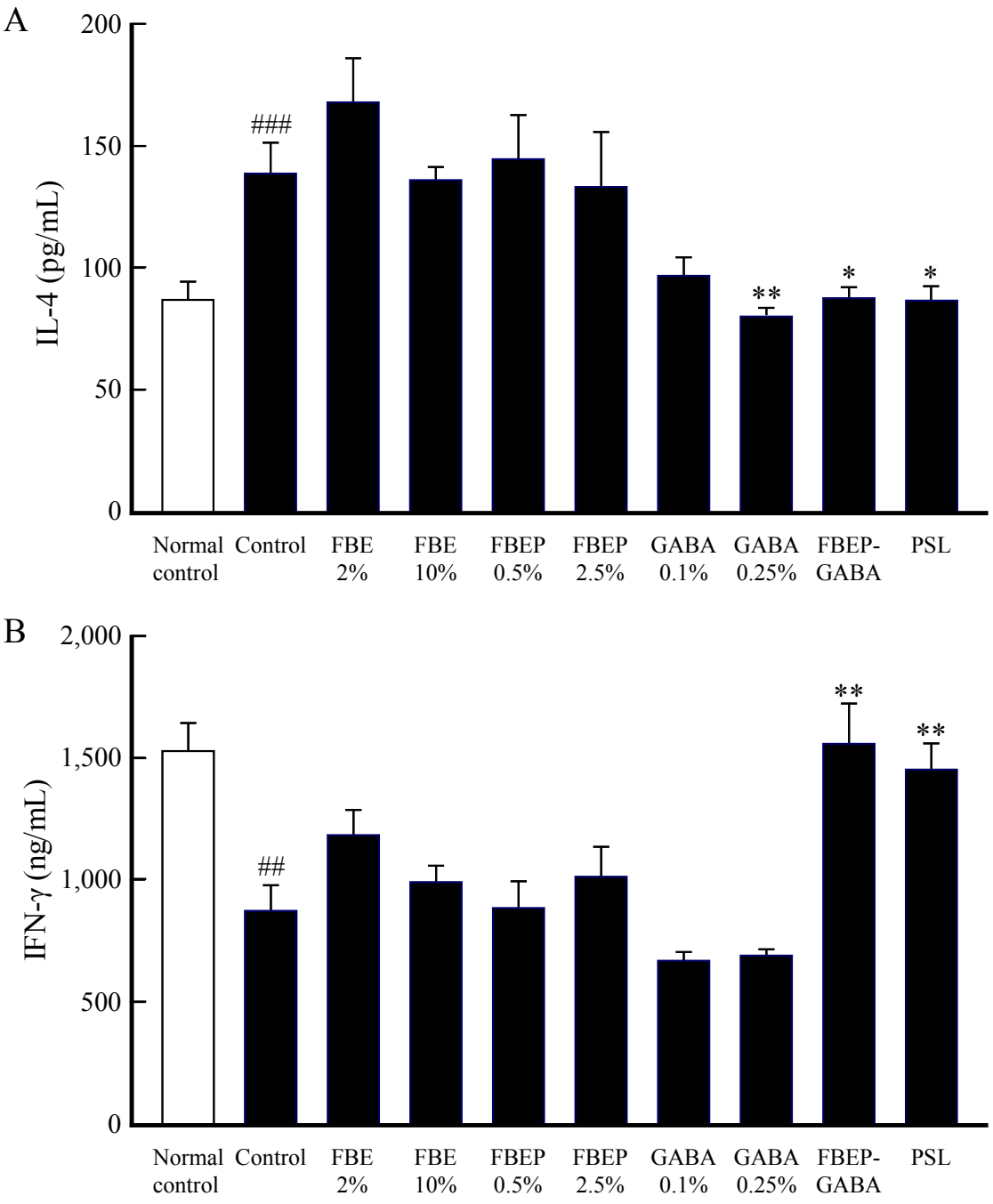


**Fig. 3-2. Effects of test substances on cytokine, IL-4 (A), and IFN-γ (B), production by spleen cells in NC/Nga mice.**

Splenocytes were prepared from NC/Nga mice 10 weeks after the initial challenge with PiCl. Cells ($1.2 \times 10^7$ cells/mL) were cultured in a 24-well plate with anti-CD3 mAb (2 μg/mL) at 37°C for 48 h. Each bar represents the mean ± SE for eight mice. $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$, significant difference as compared to the normal control group. $^{*}p<0.05$ and $^{**}p<0.01$, significant difference as compared to the control group.

type or mixed type allergic reactions develop can be used to study AD. The NC/Nga mice used in the present study spontaneously develop dermal symptoms similar to human AD,[66] and it has been reported that repeated application of hapten to mice under a specific pathogen-free environment can result in the development of the same dermatitis as in spontaneous development.[67] In the present study, PiCl was used as the hapten. It was applied to the auricles of NC/Nga mice once a week for 10 weeks continuously. The development of dermatitis was accompanied by an increase in serum IgE levels and by increased IL-4 and decreased IFN-γ levels in the splenocytes. According to a report by Gao *et al.*,[68] it was confirmed that continuous application of mite antigens to the auricles of NC/Nga mice once a week for 4 weeks continuously resulted in increased serum IgE levels and in increased IL-4 and decreased IFN-γ mRNA expression levels in the auricular tissue. We believe that their report corresponds well to the results of the present study.

Although single administration of each of the test substances (FBE, FBEP, GABA) to AD-like skin lesions resulted in thickened auricles during the study period, the auricles were significantly and strongly reduced in thickness as compared to the PiCl-treated control group, with strength of reduction in the order of FBEP > GABA > FBE. Since GABA was observed to reduce the increase in serum IgE and splenocyte IL-4 production, it was thought that adjustment of the Th1/Th2 balance to a Th1-predominant position reduced the AD-like skin lesions. However, since IFN-γ was unaffected by single administration of GABA, the skin barrier function, improved by

promoting the production of hyaluronic acid in the dermal fibroblasts, might be one of the causes of the decrease in ear thickness. On the other hand, the FBE and FBEP did not clearly influence serum IgE, IL-4, or IFN-γ, reducing only auricle thickness, T-cells do not appear to be the target of the anti-inflammatory action of FBE and FBEP. It has been reported that several plant polyphenols reduce the release of inflammation-inducing substances.[69] Furthermore, it is thought that the anti-inflammatory effect of genistein in NC/Nga mice is due to suppression of chemical mediators released from mast cells as a key effector cell in early-phase allergic inflammation,[70] and a similar effect can be also expected for the barley polyphenols contained in FBE and FBEP.

We examined the influence of combined administration of FBEP and GABA upon AD-like skin lesions but were unable to confirm any additive/synergistic effects on ear thickness reduction. However, only with combined administration of FBEP and GABA was a normalizing effect observed for IFN-γ in splenic cells. We confirmed an increase in erythrocyte GPx activity as well as an increased effect on the hepatic GSH level in the mixed feeding test of FBEP using normal mice. On the other hand, it has been reported that antigen-presenting cells (APCs) contain abundant levels of reduced-type GSH and produce large amounts of IL-12, which can induce differentiation of naive T cells into the Th1 type.[71,72] The existence of a positive feedback mechanism has been further confirmed, in which IFN-γ, a Th1 cytokine, increases the ratio of intracellular GSH/GSSG, while IL-4, a Th2 cytokine, decreases this ratio.[73] In

other words, it is possible that in the case of combined administration of FBEP and GABA, in addition to control of the Th1/Th2 balance by GABA, the effect of FBEP on the intracellular GSH redox state influences the Th1/Th2 balance to produce Th1 dominant status. In order to confirm this, it is necessary to investigate the influence of FBEP on GSH content and production of IL-12 by APC.

We have obtained an interesting finding on the combined administration of FBEP and GABA in a human study. In the open human study, following intake of a drink containing 2,000 mg of FBEP and 150 mg of GABA for 4 weeks continuously, the subjects were confirmed to have significantly higher levels of secretory IgA (s-IgA) in the saliva as compared to the level before intake.[74] It has been suggested that s-IgA in the saliva is a major immunological factor in preventing the invasion of microorganisms and macro-molecular foreign substances (antigens) into mucosal areas, and that s-IgA in the saliva in a patient exhibiting pollinosis or perennial rhinitis is clearly lower than in a healthy person, and that a low s-IgA level is potentially related to allergic sickening.[75]

Based on a comparison of the thickness-reducing effect of FBE and FBEP, the anti-inflammatory substances in FBE are thought to have been concentrated to FBEP, but the ingredients related to the anti-inflammatory effects of FBEP are not yet known. Further examination is necessary to identify the ingredients involved in FBEP, including the mechanism of anti-inflammatory action with respect to combined administration of FBEP and GABA.

With respect to body-weight changes during the study period,

similar normal body-weight increases, as in the normal control group, were observed in all groups for the three substances tested (FBE, FBEP, GABA). Hence it is suggested that these three substances are very safe materials, even for administration as long as 10 weeks. On the other hand, in the PSL group a significant reduction in the increase in body weight was observed. There were hardly any differences in the amounts of intake among the groups, the suppressed body weight gain is not thought to be related to these administrations. A similar, reduced body weight gain was also confirmed in the report of Hirasawa *et al.*,[76] although the administration route was by application.

The present study confirms that FBEP and/or GABA reduced AD-like skin lesions in NC/Nga mice. It is suggested that combined administration of FBEP and GABA causes an increase in serum IgE and splenocyte IL-4 and a decrease in splenocyte IFN-γ, having adjusted the Th1/Th2 balance to a Th1 dominant status. Thus it is to be expected that simultaneous intake of FBEP and GABA alleviates allergic symptoms such as AD.

## 3.5 Summary

We examined the effects single and combined administration of fermented barley extract P (FBEP), prepared from barley-*shochu* distillery by-products, and γ-aminobutyric acid (GABA) on the development of atopic dermatitis (AD)-like skin lesions in NC/Nga mice. Single administration of FBEP and GABA dose-dependently reduced the development of AD-like skin lesions in mice. GABA

reduced the development of AD-like skin lesions by suppressing serum immunoglobulin E (IgE) and splenocyte interleukin (IL)-4 production, while FBEP reduced skin lesions without affecting the IgE or cytokine production. However, in mice with induced AD-like skin lesions, combined administration of FBEP and GABA decreased serum IgE levels and splenic cell IL-4 production, and increased splenic cell interferon-γ production. These results suggest that combined administration of FBEP and GABA alleviated AD-like skin lesions in the NC/Nga mice by adjusting the Th1/Th2 balance to a Th1-predominant immune response.

# Chapter 4
# Anti-hyperuricemic Effect of Fermented Barley Extract is Associated with Increased Urinary Uric Acid Excretion.

## 4.1 Introduction

Gout and hyperuricemia are considered to be lifestyle-related diseases, in which environmental factors, in addition to the hereditary background, are largely associated with their incidences. Hyperuricemia is also an independent predictive factor of metabolic syndrome[77] and is often accompanied by lifestyle-related diseases, such as hypertension, abnormal glucose tolerance, hyperlipemia, obesity, *etc*. Therefore, for the prevention and treatment of gout and hyperuricemia, it is important to not simply control serum UA levels, but also provide preventative lifestyle advice, such as diet and exercise therapy. Among the options for the prevention and treatment of hyperuricemia, limiting calorie intake as a diet therapy is one of the most popular methods. However, it is not necessarily easy to maintain the strict, limited calorie intake for an extended period of time. For gout and hyperuricemia, in which early detection and treatment are important, it is advisable to control the level of UA, even for individuals with only slightly elevated serum UA (who may not satisfy the criteria for pharmacotherapy) or for mild hyperuricemia. In this context, there is an increasing need for food enabling the control of UA levels. For example, the protein in milk products has been reported to lower serum UA levels by increasing the amount of UA excretion into the urine.[78] It has also been reported that several

flavonoids, which have an inhibitory effect on xanthine oxidase (XOD) activity, are able to reduce serum UA levels by inhibiting UA biosynthesis.[79,80] In addition, several dietary fibers have been reported to reduce the increase of serum UA by inhibiting absorption of dietary nucleic acid.[81,82]

It has been reported the effects of FBE on preventing fatty liver induction by orotic acid.[3,4] Abnormalities in purine and pyrimidine metabolism are believed to be a cause for fat accumulation in the liver of rats fed orotic acid.[7] Furthermore, serum UA levels were significantly reduced after continuous intake of FBEP for 12 weeks in an exploratory open study. The FBEP dose in the clinical study was approximately 2,000 mg/day (dry weight basis), and its components consisted of a wide range of proteins, polyphenols, and dietary fibers. As a result, the components involved in reducing serum UA levels, as well as their detailed mechanism of action, have not yet been clarified.

This chapter aimed to investigate the mechanism involved in lowering human serum UA by FBEP treatment, and to establish an *in vivo* screening model of the components involved, using three hyperuricemia rat models.

## 4.2 Materials and Methods

*Experimental design* This study was composed of three separate experiments. We first examined the effect of FBEP on rats with oxonic acid-induced hyperuricemia (Exp. 1). Next, we investigated the effect of FBEP given to rats with high nucleic acid feed-induced

hyperuricemia (Exp. 2). Finally, we investigated the influence of FBEP on adenine-induced hyperuricemic rats (Exp. 3). The animal experiments were performed in accordance with the "Standards Relating to the Care and Management, etc. of Experimental Animals (Notification No. 6, March 27, 1980, of the Prime Minister's Office, Japan, revised May 28, 2002)" and "Japan's Act on Welfare and Management of Animals (up to the revisions of Act No. 50 of 2006)".

*Materials* Allopurinol, potassium oxonate and adenine were purchased from Sigma-Aldrich (St. Louis, MO). Inosinic acid was purchased from Kyowa Hakko Kogyo (Tokyo). Water for injections and sodium carboxymethyl cellulose (CMC) were purchased from Otsuka Pharmaceutical Factory (Tokushima, Japan), and Junsei Chemical (Tokyo), respectively. All other reagents were purchased from Wako Chemical (Tokyo). The preparative procedure of FBE and FBEP are described in Chapter 2 and 3, respectively. FBE was concentrated by vacuum evaporation. FBE concentration was measured as Brix values by a portable refractometer (PAL-1; Atago, Tokyo). Brix 8 FBE obtained from barley-SDB was concentrated to Brix 60.

*Animals* Four-week-old male SD rats for Exp. 1, and 5-week- and 4-week-old male Wistar rats (SLC Japan, Shizuoka) for Exp. 2 and 3, respectively, were subjected to experimentation after a one-week period of quarantine and domestication. In order to make the average body weight and standard deviations almost equal among the groups, the rats were classified into the following groups: For Exp. 1 and 2, each group consisted of 8 rats, and for Exp. 3, the

untreated group consisted of 6 rats and the other groups consisted of 10 rats. Individual identification of the rats was accomplished by applying picric acid to the body hair. Rats were housed 2 per stainless steel cage and the following conditions were set for the animal room: temperature, 21-27°C; relative humidity, 40-70%; ventilation, 18 times/h; and lighting, 12 h (7:00 to 19:00). The rats were allowed food and water *ad libitum*.

*Diet* In Exp. 1, the rats were fed a MF solid diet (Oriental Yeast, Tokyo). In Exp. 2, the rats were fed either a MF-powdered basal diet or a high nucleic acid diet, with 20% inosinic acid added to the basal diet. In Exp. 3, the rats were fed a MF-powdered basal diet and received no treatment, or received a diet containing 0.75% adenine.

*Analytical procedures* In experiment 1, the effect of FBEP on UA biosynthesis was investigated.

1) Group configuration: The experiment consisted of five groups: a control group, three FBEP administration groups (dose: 1700, 3400, or 6800 mg/kg), and an allopurinol administration group (dose: 10 mg/kg).
2) Administration method: The control and FBEP groups were given water or an FBEP suspension every day from day 0 to day 7. The allopurinol group was given 0.5% CMC from day 0 to day 6 and an allopurinol suspension 1 h after oxonic acid administration on day 7. The fluids (10 mL/kg each) were given by forced oral administration using a sonde tube for rats.
3) Hyperuricemia induction method: On day 7, a hypodermic

injection of potassium oxonate suspension, adjusted to 25 mg/mL in 0.5% CMC, was given 1 h after the administration of the test samples. The volume of the suspension was 10 mL/kg (dose, 250 mg/kg). All rats were deprived of feed after the evening of day 6.

4) Body weight measurement: Individual body weights were measured at day 0, 4 and 7.
5) Serum UA: After 3 h of oxonic acid treatment, rats were anesthetized with diethyl ether and blood was collected from the abdominal aortic vessel, and serum preparation was performed. Serum UA levels were measured using a commercial kit (Uric Acid C-Test, Wako).

In experiment 2, the effect of FBE and FBEP on the absorption of nucleic acid was investigated.

1) Group configuration: The experiment consisted of five groups: the untreated group, control group, two FBEP administration groups (dose: 2,000 or 4,000 mg/kg), and an allopurinol administration group (dose: 10 mg/kg).
2) Administration method: Injection water, FBEP suspensions, and an allopurinol suspension were given to the control, FBEP, and allopurinol groups every day from day 0 to day 14, respectively. The fluids (10 mL/kg each) were given by forced oral administration using a sonde tube for rats.
3) Hyperuricemia induction method: Rats were fed a MF diet containing 20% inosinic acid *ad libitum* between days 7-14.
4) Body weight measurement: Individual body weights were measured at days 0, 4, 7, 11 and 14.

5) Food consumption: The amounts of residual feed and provided feed were measured on the day of body weight measurement to calculate the daily food intake per individual for each cage.
6) Urine volume and pH: The rats from each group, except the untreated group, were transferred to metabolism cages on the evening of day 13 until the morning of day 14 for urine collection, and the volume of collected urine was measured using a measuring pipette. All the rats of the untreated group were transferred to metabolism cages on the evening of day 17 until the morning of day 18 for urine collection, and urine volumes were measured as above. Fresh urine from day 13 was used for pH measurement with a test paper (Uropaper III Eiken, Eiken Chemical, Tokyo).
7) Serum UA: On day 14, rats were anesthetized with diethyl ether prior to collection of blood samples from the caudal artery for serum isolation. An aliquot of 200 μL of serum was mixed with 25 μL 4 mol/L perchloric acid. The mixture was then mixed with 20 μL 4 mol/L potassium dihydrogen phosphate, centrifuged (20,000×g, room temperature, 10 min), and the supernatant was collected for UA measurement. UA concentration was measured by HPLC: column, Sim-Pack CLC-$NH_2$ ($\phi$ 6.0 mm × 150 mm, Shimazu, Kyoto); mobile phase, $CH_3CN$/20 mM $KH_2PO_4$ = 3/2 (v/v).
8) Serum and urinary allantoin: Serum and urine samples taken on day 14 were measured for allantoin concentration by HPLC: column, Atlantis HILIC Silica ($\phi$ 4.6 mm × 150 mm, Waters, Milford, MA); mobile phase, $CH_3CN/H_2O$ = 95/5 (v/v). The serum and urine samples were diluted two-fold with distilled

water and centrifuged (20,000×g, room temperature, 10 min) to obtain supernatants, which were then passed through an ultrafiltration membrane (Microcon Ultracel YM-10 Regenerated Cellulose 10,000 MWCO, Millipore, Bedford, MA). Three volumes of $CH_3CN$ were added to each ultrafiltrate and the mixtures were centrifuged (20,000×g, room temperature, 10 min) to obtain supernatants for HPLC.

In experiment 3, the effect of FBE and FBEP on UA excretion was investigated.

1) Group configuration: The experiment consisted of five groups: an untreated group, control group, two FBEP administration groups (dose: 2,000 or 4,000 mg/kg), and the FBE administration group (dose: 6,500 mg/kg).
2) Administration method: The untreated and control groups were administered water injections, FBEP and FBE groups were administered each soil suspension from day 0-14. The administered volume of the fluids was 10 mL/kg each time. An oral sonde for rats was employed for forced administration twice a day.
3) Hyperuricemia induction method: Adenine was mixed with the feed at a ratio of 0.75%, which was freely accessible from day 0 to day 14.
4) Body weight measurement: Individual body weights were measured at days 0, 4, 7, 11 and 14.
5) Food consumption and water intake: The amounts of residual food and water, and the amounts of provided food and water, were

measured on the day of body weight measurement to calculate daily intake of food and water per individual.

6) Urine volume: All rats were transferred to metabolism cages at 10:00 am on day 14 to collect urine for 6 hours up to 16:00. The volume of the collected urine was measured with a measuring pipette. Rats transferred to metabolism cages were deprived of water and feed.
7) Urine pH: Ames urine test paper (Siemens Medical Solutions Diagnostics, Tokyo) was used to measure the pH of fresh urine on day 14.
8) Serum or urinary UA, allantoin, creatinine and urea nitrogen: Rats were anesthetized with diethyl ether on day 15 to take blood samples from the ventral aorta, and the blood samples were centrifuged to obtain serum samples. The urine samples consisted of supernatants prepared by centrifuging urine collected on day 14. The urine samples were diluted with 3 volumes of distilled water for the measurement of urinary UA concentration. Five volumes of $CH_3CN$ were added to each of the urine samples and the mixtures were centrifuged (16,000×*g*, room temperature, 10 min) and the supernatants were used for the measurement of urinary allantoin concentration. UA and allantoin concentrations of the sera and urine were measured by the same methods as used in Exp. 2. Urea nitrogen concentrations of the sera and urine were measured using a B-Test (Wako, Osaka, Japan) for urea nitrogen, and allantoin concentrations of the sera and urine were measured using a Creatinine-Test (Wako).

9) UA clearance: Based on measurements of serum UA concentration, urinary UA concentration, and urine volume, the following equation was used to calculate UA clearance ($Cl_{ur}$).

$$Cl_{ur}(mL/kg/min)=U_{ur}(mg/mL)\times V(mL/kg/min)/P_{ur}(mg/mL)$$

$P_{ur}$: Serum UA (mg/mL)

$U_{ur}$: Urinary UA (mg/mL)

V: Urine volume/kg body weight/min (mL/kg/min)

*Statistical Analyses* Dunnett's test, U test, and Kruskal-Wallis test were used to test statistically significant differences between the control group and the test groups. For Exp. 2 and 3, Student's *t*-test and U test were used to test statistically significant differences between the control group and the untreated group. A significance level of 5% was adopted.

## 4.3 Results

*Exp. 1* Although the data is not given in the tables, the average body weight on days 0, 4, and 7 were approximately 140 g, 170 g, and 200 g, respectively. There were no significant differences among the groups.

*Serum UA* Table 4-1 shows serum UA concentration. The FBEP groups tended to be lower in serum UA concentration than the control group, but no significant differences were observed. In contrast, serum UA concentration was significantly lower in the allopurinol group, a positive control, than in the control group ($p<0.001$).

*Exp. 2* Body weight gain was –31% in the high-dose FBEP group and –86% in the allopurinol group when compared with the control

group ($p<0.05$ and $p<0.001$, respectively). Food consumption was –13% in the high-dose FBEP group and –32% in the allopurinol group when compared with the control group ($p<0.05$ and $p<0.01$, respectively).

*Serum UA and allantoin* Table 4-2 gives the serum UA and serum allantoin concentrations. The serum UA concentration was significantly higher in the control group than in the untreated group ($p<0.001$), indicating that the high-inosinic acid feed induced hyperuricemia. Serum UA was significantly lower in the allopurinol group than in the control group ($p<0.001$), but there were no significant differences between the FBEP groups and the control group. As was the case with the serum UA concentration, the serum allantoin concentration was significantly lower in the untreated and allopurinol groups than in the control group. In contrast, there were no significant differences between the FBEP groups and the control group.

*Urinary volume and pH* Urine volume was significantly smaller in the untreated group than in the control group ($p<0.001$). However, there were no significant differences in urine volume among the groups given the high-inosinic acid feed. Urine pH was significantly higher in the allopurinol group than in the control group ($p<0.01$). Urinary allantoin concentration was significantly higher in the control group than in the untreated group ($p<0.001$). In contrast, there were no significant differences among the groups given the high-inosinic acid feed.

**Table 4-1. Effect of FBEP on serum uric acid levels in rats with oxonic acid-induced hyperuricemia (Exp. 1)**

| | Group | | | | |
|---|---|---|---|---|---|
| | Control | FBEP (LD) | FBEP (MD) | FBEP (HD) | Allopurinol |
| Serum uric acid (mg/dL) | 5.3 ± 0.7 | 5.1 ± 0.6 | 4.5 ± 0.6 | 4.7 ± 0.7 | 2.7 ± 0.2 |
| *P* value (vs control) | — | NS | 0.059 | NS | <0.001 |

FBEP, fermented barley extract P; LD, low-dose; MD, medium-dose; HD, high-dose.
Values are expressed as means ± SD.
The *p*-values were calculated according to Dunnett's test.

**Table 4-2. Serum uric acid and allantoin concentrations of rats fed a high nucleic acid diet (Exp. 2)**

| | Group | | | | |
|---|---|---|---|---|---|
| | Untreated | Control | FBEP (LD) | FBEP (HD) | Allopurinol |
| Uric acid (mg/dL) | 0.5±0.1$^{***}$ | 1.3±0.2 | 1.3±0.1 | 1.2±0.2 | 0.3±0.2$^{***}$ |
| Allantoin (mg/dL) | 1.7±0.1$^{***}$ | 16.5±3.9 | 17.4±3.9 | 20.6±4.8 | 10.3±5.0$^{*}$ |

FBEP, fermented barley extract P; LD, low-dose; HD, high-dose.
Values are expressed as means ± SD.
Significantly different from the value of rats fed a control diet, $^{*}p<0.05$, $^{***}p<0.001$.

*Urinary allantoin excretion* Fig. 4-1 shows the amount of allantoin excreted into the urine per 100 g body weight. The amount of urinary allantoin excretion was significantly higher in the high-dose FBEP group than in the control group ($p<0.05$).

*Exp. 3* Body weight gain, food consumption, and water intake of rats fed an adenine diet are shown in Table 4-3. As expected, the untreated group increased in body weight during the experiment. In contrast, all adenine groups, including the control group, showed either an intense suppression in body weight increase or body weight decrease, which is believed to have resulted from adenine administration. However, the high-dose FBEP and FBE groups had significantly higher weight gains than the control group ($p<0.05$). The amount of food consumption by all the adenine groups, including the control group, was small throughout the administration period. The amount of food consumption by the high-dose FBEP group was slightly elevated compared with the control group. Water intake by the control group was significantly higher than the untreated group ($p<0.001$). The high-dose FBEP and FBE groups consumed significantly more water than the control group ($p<0.01$ and $p<0.05$, respectively).

Urinary volume and pH of rats fed an adenine diet are shown in Table 4-4. Urinary volume of the control group showed the tendency to be high as compared with the untreated group ($p=0.054$). Urinary volume was significantly higher in the high-dose FBEP and FBE groups than in the control group ($p<0.01$ and $p<0.05$, respectively). Urinary pH was significantly lower in the control group than in the

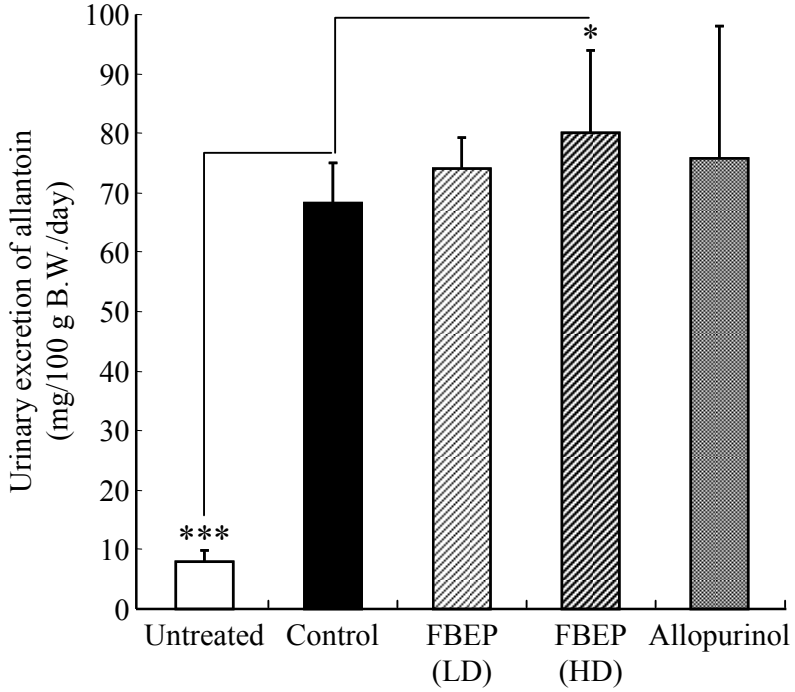


**Fig. 4-1. Urinary allantoin excretion in rats fed a high nucleic acid diet (Exp. 2).**

FBEP, fermented barley extract P; LD, low-dose; HD, high-dose.
Values are expressed as means ± SD.
Significantly different from the value of rats fed a control diet, $^{*}p<0.05$, $^{***}p<0.001$.

**Table 4-3. Body weight gain, food consumption and water intake in rats fed an adenine diet (Exp. 3)**

| | Group | | | | |
|---|---|---|---|---|---|
| | Untreated | Control | FBEP (LD) | FBEP (HD) | FBE |
| Body weight gain (g/14 days) | 79.1±12.2$^{***}$ | −14.1±6.3 | −10.2±6.7 | −3.3±4.3$^{**}$ | −6.7±6.3$^{*}$ |
| Food consumption (g/rat/day) | 14.2±1.3$^{***}$ | 5.0±0.8 | 5.2±0.8 | 5.5±0.9 | 5.2±0.7 |
| Water intake (mL/rat/day) | 17.9±1.1$^{***}$ | 24.0±3.0 | 25.1±3.1 | 27.7±3.8$^{**}$ | 26.8±3.3$^{*}$ |

FBEP, fermented barley extract P; LD, low-dose; HD, high-dose; FBE, fermented barley extract.
Values are expressed as means ± SD.
Significantly different from the value of rats fed a control diet, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

**Table 4-4. Urinary volume and pH in rats fed an adenine diet (Exp. 3)**

| | Group | | | | |
|---|---|---|---|---|---|
| | Untreated | Control | FBEP (LD) | FBEP (HD) | FBE |
| Volume (mL) | 4.2±0.7 | 5.2±1.3 | 5.4±0.8 | 7.2±1.3$^{**}$ | 6.7±1.5$^{*}$ |
| pH | 8.4±0.2$^{***}$ | 7.6±0.4 | 7.5±0.6 | 7.6±0.4 | 7.3±0.3 |

FBEP, fermented barley extract P; LD, low-dose; HD, high-dose; FBE, fermented barley extract.
Values are expressed as means ± SD.
Significantly different from the value of rats fed a control diet, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

untreated group ($p$<0.001). There were no significant differences in urinary pH between the control group and other test groups.

Serum or urinary UA, allantoin, creatinine and urea nitrogen are shown in Table 4-5. There was no significant difference in serum UA concentration between the control group and the untreated group. The FBEP group showed no significant difference in serum UA concentration from the control group; however, the FBE group was significantly lower ($p$<0.05). The control group was significantly higher than the untreated group in serum allantoin, creatinine, and urea nitrogen concentrations ($p$<0.01). On the other hand, none of the groups administered the test materials showed a significant difference from the control group. The control group was significantly lower in urinary UA concentration than the untreated group ($p$<0.01). The high-dose FBEP group was significantly higher in urinary UA concentration than the control group ($p$<0.05). The control group tended to be lower in urinary allantoin concentration than the untreated group ($p$=0.095), and none of the test groups showed a significant difference from the control group. The control group was significantly lower in urinary creatinine and urea nitrogen concentrations than the untreated group ($p$<0.01). None of the test groups showed a significant difference from the control group.

Fig. 4-2 shows $Cl_{ur}$ values. The control group was significantly lower in $Cl_{ur}$ than the untreated group ($p$<0.01). Only the high-dose FBEP group was higher in $Cl_{ur}$ than the control group ($p$<0.01).

**Table 4-5. Serum or urinary uric acid, allantoin, creatinine and urea nitrogen levels in rats fed a 0.75% adenine diet (Exp. 3)**

| | Group | | | | |
|---|---|---|---|---|---|
| | Untreated | Control | FBEP (LD) | FBEP (HD) | FBE |
| Serum (mg/dL) | | | | | |
| Uric acid | 1.3±0.2 | 1.5±0.4 | 1.5±0.4 | 1.3±0.5 | 1.0±0.3* |
| Allantoin | 1.8±0.4** | 18.9±7.4 | 19.3±7.6 | 16.9±6.3 | 17.7±7.1 |
| Creatinine | 0.8±0.0** | 2.6±0.3 | 2.7±0.4 | 2.6±0.2 | 2.5±0.3 |
| Urea nitrogen | 23.1±1.3** | 170.9±16.2 | 165.1±16.3 | 158.1±18.0 | 163.1±14.3 |
| Urine (mg/dL) | | | | | |
| Uric acid | 17.2±2.4** | 1.7±0.4 | 1.4±0.4 | 2.2±0.4* | 1.3±0.4 |
| Allantoin | 132.8±76.2 | 79.4±44.3 | 92.7±48.8 | 88.4±52.4 | 92.0±46.8 |
| Creatinine | 51.2±6.9** | 12.7±1.0 | 13.6±1.5 | 12.6±1.5 | 13.7±1.4 |
| Urea nitrogen | 1137.8±183.0** | 581.1±67.1 | 572.2±45.5 | 557.6±40.0 | 601.4±55.2 |

FBEP, fermented barley extract P; LD, low-dose; HD, high-dose; FBE, fermented barley extract.
Values are expressed as means ± SD.
Significantly different from the value of rats fed a control diet, $^{*}p<0.05$, $^{**}p<0.01$.

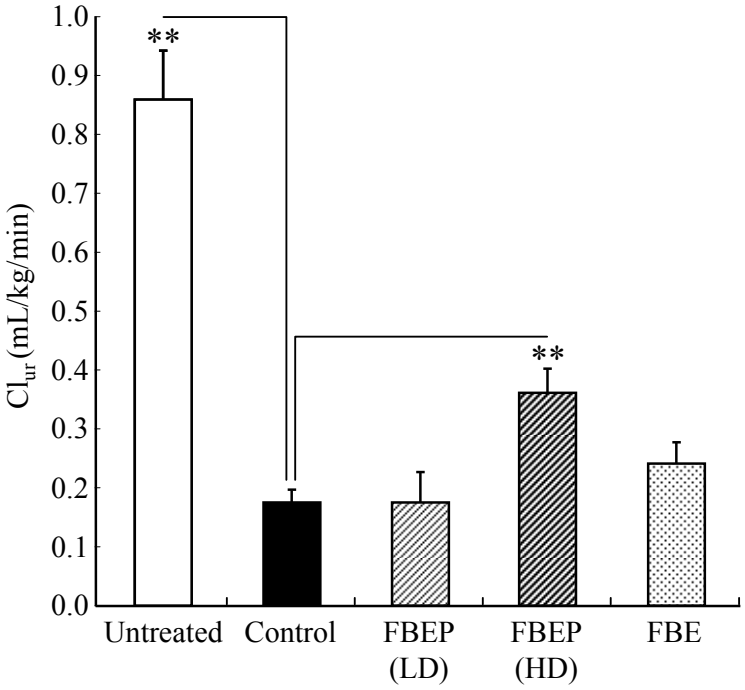


**Fig. 4-2. Uric acid clearance ($Cl_{ur}$) in rats fed a 0.75% adenine diet (Exp. 3).**

FBEP, fermented barley extract P; LD, low-dose; HD, high-dose; FBE, fermented barley extract.
Values are expressed as means ± SD.
Significantly different from the value of rats fed a control diet, $^{**}p<0.01$.

## 4.4 Discussion

The process of metabolizing intracellular purines such as adenosine and guanosine, and that of decomposing xanthine into UA, are found to exist in all living things. However, some groups of enzymes involved in decomposing UA into substances more water-soluble may have been partially inactivated. Therefore, the final metabolites from a purine metabolizing process may differ by species. In Anthropoidea, including humans, uricase is inactivated and the final metabolite from purine metabolism is UA.[83-86] On the other hand, in rodents such as mice and rats, which belong to the same polyphagia as humans and are frequently used in animal tests, UA is metabolized by uricase, a UA-oxidizing enzyme, into allantoin and excreted in urine.[87] Accordingly, we prepared hyperuricemia model-rats by administering the uricase inhibitor oxonic acid (Exp. 1), and examined the effect of FBEP on reducing serum UA levels. Since the serum UA value of five-week-old male SD rats is 0.49-0.74 mg/dL,[88] it is thought that serum UA of the control group increased approximately 10-fold compared to untreated rats. As a result, the group administered the potent UA production inhibitor allopurinol exhibited a remarkable reduction in serum UA levels; whereas, similar reductions in serum UA levels were not observed in the group administered FBEP. Therefore, the effects of FBEP on serum UA levels observed in humans was not reproduced in this animal model. In addition, as a result of the *in vitro* examination on the effect of FBEP upon XOD, a metabolic enzyme for purine, no inhibitory effect on XOD was observed at concentrations below 1000 μg/mL (data not

shown). Consequently, it is considered that FBEP may not substantially inhibit the biosynthesis of UA.

In Exp. 2, we examined the effect of FBEP using rats administered a high dose of nucleic acid. As a consequence, in comparison with the control group, none of the FBEP-dose groups showed a statistically significant, lowering effect on serum UA concentrations. However, for the group administered a high dose of FBEP, the body-weight converted values of the excreted amount of allantoin in urine showed significantly higher values as compared to the control group. A dose-dependent relationship between FBEP administration and UA excretion was not observed. It is thought that the lowest dosage in which a UA excretion promotion effect is observed was the administration level of the FBEP high-dose group. The elevated serum and urine allantoin concentrations indicated that the metabolic amount of nucleic acid increased due to the feed containing a high dose of inosinic acid, but subsequently UA metabolism occurred *via* a metabolic route not existing in humans. In other words, FBEP may not inhibit absorption of nucleic acid in the digestive tract.

Based on the results of Exp. 1 and 2, the mechanism of FBEP in lowering serum UA levels may be the promotion of UA excretion in urine. Thus, for Exp. 3, we designed a study using rats fed an adenine diet, with the hyperuricemia characterized as the decreased excretion-type.[89,90] As a result, the serum UA levels in the group administered FBE, as a sample of FBEP in the pre-purification stage, showed significantly lower values compared with the control group, while the high-dose FBEP group, although the effect was much

weaker than in the FBE group, showed a lowering tendency compared with the control group. In the analysis of the UA concentration in urine, the FBEP high-dose group showed significantly high values compared with the control group. A possible reason why serum UA did not decrease significantly in the FBEP high-dose group is that there may have been differences in the metabolism of UA to allantoin. That is, the serum allantoin of FBEP high-dose group is the lowest. In addition, it has been reported that the serum allantoin level of rats fed an adenine diet was remarkable increased, whereas the serum UA level did not change considerably.[91] Yokozawa *et al*. consider that the excessive UA was metabolized to allantoin in order not to raise UA levels in the body beyond a certain level. Although Exp. 3 was used as a decreasing excretion model of UA, it may be applicable as a model of lowered UA excretion-type hyperuricemia by inhibiting uricase. Furthermore, as the FBEP high-dose group showed significantly elevated levels in both water intake and urine production compared with the control group, FBEP appears to be able to further reduce elevated serum UA concentrations by excreting a comparatively large amount of urine containing relatively high UA levels. In addition, by computing and comparing $Cl_{ur}$, the action by which FBEP improved UA excretion ability, which declined by adenine diet, became clearer. On the other hand, FBEP or FBE treatments were unable to significantly alter the effect of adenine administration on indicators of renal disorders (serum urea nitrogen and creatine concentrations) and severe renal toxicity was confirmed in all adenine-administered groups.

The mechanism involved in the promoting action of FBEP on the UA excretion requires further study, and it will be necessary to investigate what effect FBEP may have upon the UA transporter responsible for the re-absorption of UA from renal tubules, such as URAT 1.[23)]

The present findings suggest that the mechanism involved in the lowering effect of FBEP on serum UA levels may be attributed to the promotion of urinary UA excretion. In conclusion, it is thought that the adenine-loaded rat model, producing lowered UA excretion-type hyperuricemia, is the most appropriate *in vivo* screening model for examining the active components in FBEP and their involvement in lowering serum UA levels in humans.

## 4.5 Summary

Effects of a fermented barley extract P (FBEP) on uric acid (UA) metabolism in three hyperuricemia rat models were studied. First, the influence of FBEP on UA biosynthesis was examined using rats hypodermically administrated oxonic acid. Then, the influence of FBEP on dietary purine absorption was examined using rats fed a high dose of inosinic acid. Finally, the influence of FBEP on UA excretion was examined using adenine-administered rats. From the first two models, FBEP was found to be rarely, or not at all, involved in the biosynthesis of UA or absorption of nucleic acid. On the other hand, in the rats loaded with adenine, it was shown that the urinary UA of the high-dose FBEP group (4,000 mg/kg/day) was significantly elevated by 27% compared to the control group. In addition, the UA

clearance value in the high-dose FBEP group was increased 2-fold compared to the control group. These results suggest that FBEP reduces serum UA by increasing urinary excretion of UA.

# Chapter 5
# Effects of a Fermented Barley Extract on Subjects with Slightly High Serum Uric Acid or Mild Hyperuricemia.

## 5.1 Introduction

The number of cases in Japan of gout and its cause, hyperuricemia, have been increasing yearly as a result of the adoption of westernized and Americanized diets. The incidence of hyperuricemia, the underlying disease of gout, has been reported to be approximately 20% of Japanese male adults.[25,26] Even though there have been few epidemiological studies, the incidences of gout and hyperuricemia have also been increasing worldwide with changes in environmental factors.[92,93] The Japanese Society of Gout and Nucleic Acid Metabolism defines hyperuricemia as a condition with over 7.0 mg/dL of serum UA, irrespective of the gender and age. In the selection of methods and medicines for the treatment of hyperuricemia, it is important to classify the disease types. Hyperuricemia is classified into 3 types: the increased production type, in which UA is excessively produced; the decreased excretion type, in which the capability for UA excretion is reduced due to decreased renal function; and the mixed type, which is a combination of both the above types. Eighty-five percent of all the cases of gout and hyperuricemia in Japan involve decreased excretion of uric acid from the kidney.[94,95] It has been reported that chitosan, as a foodstuff, decreases serum UA by inhibiting the absorption of purine.[96] However, it has not been reported that a foodstuff can

effectively decrease serum UA in humans by accelerating the UA excretion.

FBEP had been found to significantly decrease the serum UA level in an open study using male Japanese subjects with a serum UA level in the range of 6.0-7.9 mg/dL.[28] In chapter 4, FBEP reduces serum UA by increasing urinary excretion of UA in the hyperuricemia rat models.

The present study was aimed at making a further, detailed examination of the effectiveness and safety of FBEP. We conducted a double-blind study of Japanese men and women with slightly elevated serum UA levels, but who did not require treatment for this.

## 5.2 Materials and Methods

*Subjects* The test was carried out on Japanese men and women with a serum UA level of 6.0-7.9 mg/dL and an age range of 20-65 years. The exclusion criteria were as follows:

i) Those who had gout.
ii) Those who made regular use of medicines and/or foods that might influence the serum UA level.
iii) Those who had severe diseases such as diabetes mellitus, liver disorders, renal disorders, or cardiovascular diseases.
iv) Those who, at the examination prior to the trial, had both over urinary protein levels and positive urinary creatinine levels, or having over 2+ urinary protein levels.
v) Those who had any food allergies.
vi) Those who were already enrolled in another study at the start of

this study.

vii) Those who were pregnant, breast-feeding or expected to become pregnant during the study period.

viii) Those who were deemed unsuitable for this study by the investigators.

Prior to treatment, we performed two pre-test examinations, four weeks apart, and selected 111 subjects who met the eligibility criteria. The present test was executed while abiding with the ethical guidelines that are in accordance with the Helsinki Declaration and the Ethical Guidelines for Epidemiological Research, Japan (notified by Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labor and Welfare).

*Test food* FBEP, obtained from barley (*Hordeum vulgare f. distichon*) SDB, was evaluated in a clinical examination. The preparation of FBEP is described in Chapter 3. Food-grade instruments were used for preparing FBEP. We used a test drink containing 2 g per 30 mL for the daily intake of FBEP, and an FBEP-free placebo drink, which contained a small amount of caramel to produce the same color as the test drink, as the control. Both drinks contained equal amounts of honey, galacto-oligo saccharide, and lemon fruit juice as secondary components.

*Trial design* A randomized, placebo-controlled, parallel-group, double-blind study was carried out. The primary end point was the change in serum UA level from pre-treatment to week 12 after the treatment. The secondary endpoints were the changes in urinary UA level and UA clearance from pre-treatment to the end of the treatment

period. The test schedule is shown in Fig. 5-1. We conducted a pre-test examination about 5 weeks prior to the trial. About 4 weeks later, we conducted an additional pre-test examination, after which we selected 111 subjects who qualified for the present study. The parameters monitored during the experiment are detailed in the test schedule which continued for a total of 21 weeks and was comprised a 4-week recruitment period, 1-week pre-treatment period, 12-week treatment period, and 4-week post-experimental period. During the treatment period, the food consumption and changes in the physical conditions were recorded daily, a dietary survey for cataloging all food consumed was filled in for 3 day prior to the examination. Alcoholic beverages were not allowed in the 3 day prior to the examination, and no food or drinks, except water, were allowed 8 hours prior to the examination. Urine was collected for 24 hours prior to the examination by using U-mate P (Sumitomo Bakelite, Akita, Japan). The subjects were asked to visit the hospital without consuming the food to be used on the examination days during the treatment period. UA clearance was calculated by using the classification for disease type in the Guidelines for the Management of Hyperuricemia and Gout.[26)] The equation of Fujimoto *et al*. was used for calculating the body surface area in the formula for UA clearance rate.[97)] However, we used urine collected for 24 hours on the day before the examination as the samples in the present study, and did not follow the experimental procedure described in those guidelines.

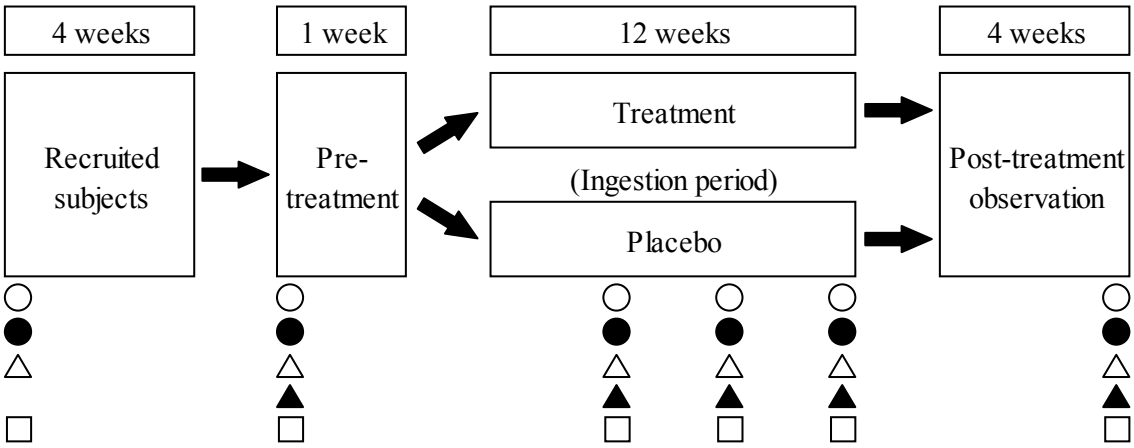


**Fig. 5-1. Design of the study.**

Unfilled circles, diagnostic interview; filled circles, physical examination; unfilled triangles, blood test; filled triangles, urine test; squares, diet survey.

*Clinical laboratory parameters*

i) Physical examination. A total of six measurements were taken for assessing the body weight (BW), blood pressure (BP) and pulse rate: once 5 weeks before treatment; once 1 week before treatment; then 4, 8, and 12 weeks during treatment; and lastly, once 4 weeks after treatment. Height (Ht) was measured only once, 5 weeks before the treatment, and was used to calculate the body-mass index (BMI).

ii) Blood and urine tests. Peripheral blood samples were monitored for the following indices: white blood cell (WBC), red blood cell (RBC), and platelet counts; hemoglobin (Hb) level; hematocrit (HMC) value; MCV, MCH, MCHC, AST, ALT, ALP, γ-GTP, LDH, total bilirubin (Tbil), fasting blood sugar (FBS), total protein (TP), and albumin (Alb) levels; A/G ratio; total cholesterol (Tcho), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and triglyceride (TG) levels; blood urea nitrogen (BUN), creatinine (Cr), uric acid (UA); and electrolytes (Na, K, Cl, Ca, and P). Qualitative urine tests assessing the pH value, glucose, protein, and occult blood levels were performed by using test paper, while the UA level was quantitatively measured. Blood and urine were determined in a clinical laboratory (SRL, Tokyo).

*Dietary survey* The intake of purine and alcoholic drinks during the study period was evaluated. The intake of purine is defined as the total purine content in the food and alcoholic drinks. Estimates of the purine content in different foods were obtained from the amounts of purine described in the Standard Tables of Food Composition in Japan (STJ)[98] and from the attached list of the

Guidelines for the Management of Hyperuricemia and Gout.[26] We used values published on websites for each company producing alcoholic drinks for the purine content in these drinks. We utilized the purine content in foods described in STJ when this information was not available on the websites. The same measurement method for purine content data was applied. The purine intake over the 3 days prior to the examination (before treatment, 4, 8, and 12 weeks during treatment, and week 4 post-treatment) was calculated from the information in the dietary survey. We define the amount of alcoholic drinks as the alcohol intake (g) calculated by the preference beverages described in STJ as the reference. The daily average of alcohol intake (g) was calculated from the data recorded in the diary.

*Statistical analysis* The serum UA levels, urinary UA levels and UA clearance rates for the test and placebo groups were compared 12 weeks after the treatment with the pre-treatment values by an analysis of covariance, in which values obtained at the examination prior to the treatment are regarded as the covariance values. The other results were evaluated by using a paired *t*-test of the presence or absence of variations at weeks 4, 8 and 12 during treatment and at week 4 post-treatment in comparison with the pre-treatment values. Each value is expressed as the mean ± SD. All statistical tests were two-tailed, a $p$ value of less than 0.05 being considered statistically significant.

## 5.3 Results

*Background details of the subjects* 468 applicants (410 men

and 58 women) between the ages of 20 and 65 years consented to participate in the study, among whom 111 subjects passed the selection criteria and were not rejected according to the exclusion criteria. However, one subject dropped out for personal reasons. Thus, the total number of subjects who completed the prescribed study schedule was 110. Among the subjects, five were unreliable with respect to the examination results and one took medication that would affect the test results. Thus, six additional subjects were excluded during the study, leaving 104 valid subjects eligible for analysis. In respect of the subject who dropped out halfway, the period from the start of treatment up to the time of dropping out was used as the evaluation period, and all the values for the clinical and physical examinations up to week 4 were used as valid data. The results of the clinical and physical examinations in week 8 after treatment were evaluated for 109 subjects, excluding one who was found to be defective in week 8 after the intake. The background of the subjects is shown in Table 5-1.

*Serum UA* The time-course characteristics for serum UA are shown in Fig. 5-2. The covariance analysis, in which the pre-treatment values were the covariates, showed a significant decrease in the test group 12 weeks after treatment ($p<0.05$). The within-group comparison showed significantly lower values for the test group in weeks 4 and 12 during treatment than the pre-treatment values (Table 5-5-2). In contrast, the placebo group did not show any significant changes.

**Table 5-1. Background details of the subjects**

| Item | Efficacy analysis | | Safety analysis | |
|---|---|---|---|---|
| | Test | Placebo | Test | Placebo |
| Number of subjects (male/female) | 54 (52/2) | 50 (44/6) | 56 (54/2) | 55 (49/6) |
| Mean age (years) | 43.3±11.3 | 43.6±9.3 | 43.5±11.2 | 42.7±9.4 |
| Mean height (cm) | 170.5±7.8 | 170.2±7.8 | 170.5±7.7 | 170.4±7.7 |
| Mean weight (kg) | 71.5±11.1 | 74.6±10.6 | 71.4±10.9 | 74.3±10.3 |

Each value is expressed as the mean ± SD.

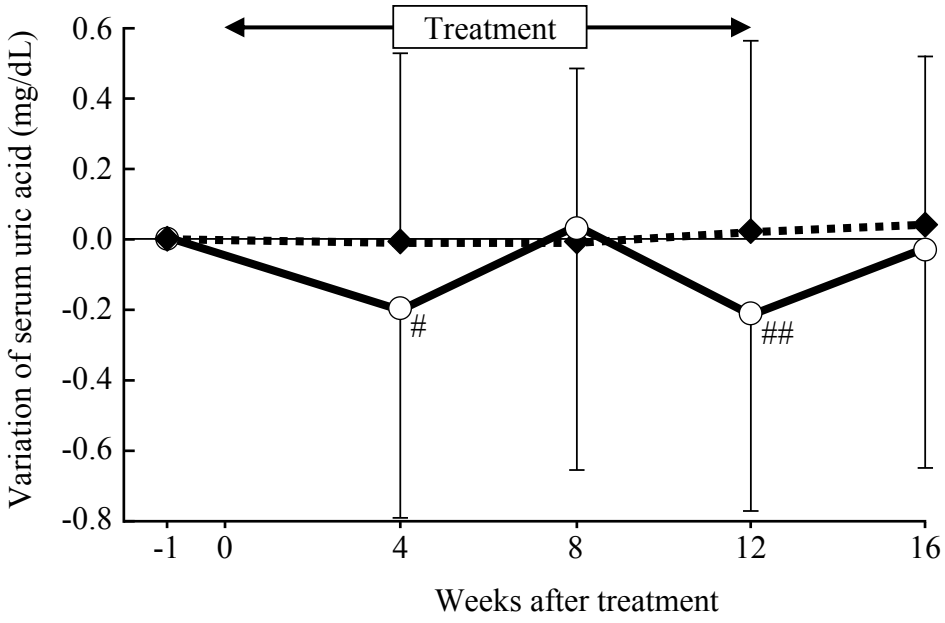


**Fig. 5-2. Time-course variation of serum uric acid.**

Unfilled circles, treatment group (n=54); filled diamonds, placebo group (n=50). Each value is expressed as the mean ± SD. $^{\#\#}p<0.05$ and $^{\#}p<0.10$, compared with the placebo group using an analysis of covariance.

*Urinary UA* The time-course characteristics for urinary UA are shown in Table 5-2. The between-groups comparison showed no significant difference in week 12. However, the within-group comparison showed significantly higher values for the test group in week 12 of treatment than the pre-treatment values. In contrast, the placebo group did not exhibit any significant changes.

*Urinary volume and pH* No significant changes were observed in either the between-groups or within-group analyses (Table 5-3).

*UA clearance* The time-course variations for UA clearance from the pre-treatment levels are shown in Fig. 5-3. The covariance analysis shows a small elevation on week 12 of treatment in the test group when compared with the placebo group ($p$=0.054).

*Physical examination* Table 5-4 shows the physical examination findings. Both the test and placebo groups showed significantly lower diastolic blood pressure in weeks 8 and 12 of the treatment period than the pre-treatment values. Nevertheless, the changes at any determined time were within the normal range and are not regarded as clinically important decreases. The placebo group exhibited a significantly lower pulse rate in weeks 4 and 8 of the treatment and in week 4 of the post-experimental period than the pre-treatment values, although this decrease did not present any clinical problem. Such other parameters as the body weight, BMI and systolic blood pressure did not exhibit any significant changes.

*Blood test* The time-course characteristics of the measurements are shown in Table 5-5-1 and Table 5-5-2. Even though some

**Table 5-2. Results for 24-hour urinary uric acid excretion**

| Item | Normal range | Group | 1 wk before treatment | 4 wks after treatment | 8 wks after treatment | 12 wks after treatment | 4 wks after termination |
|---|---|---|---|---|---|---|---|
| UUA (g/day) | 0.4-1.2 | Test | 0.607±0.204 | 0.657±0.175 | 0.639±0.238 | 0.696±0.280** | 0.641±0.249 |
| | | Placebo | 0.676±0.183 | 0.729±0.440 | 0.697±0.242 | 0.676±0.308 | 0.692±0.382 |

UUA, urinary uric acid.
Each value is expressed as the mean ± SD.
Significant differences are as follows: $^{**}p<0.01$, compared with the pre-treatment condition using the paired Student's *t*-test.

**Table 5-3. Results of urinary volume and pH**

| Item | Normal range | Group | 1 wk before treatment | 4 wks after treatment | 8 wks after treatment | 12 wks after treatment | 4 wks after termination |
|---|---|---|---|---|---|---|---|
| UV (L/day) | 0.5-2.0 | Test | 1.57±0.66 | 1.57±0.56 | 1.51±0.56 | 1.56±0.54 | 1.51±0.69 |
| | | Placebo | 1.62±0.63 | 1.57±0.66 | 1.56±0.57 | 1.63±0.63 | 1.60±0.69 |
| pH | 5.0-8.0 | Test | 5.76±0.52 | 5.83±0.61 | 5.81±0.61 | 5.80±0.51 | 5.92±0.67 |
| | | Placebo | 5.66±0.54 | 5.68±0.55 | 5.73±0.62 | 5.84±0.69 | 5.78±0.59 |

UV, urinary volume.
Each value is expressed as the mean ± SD.

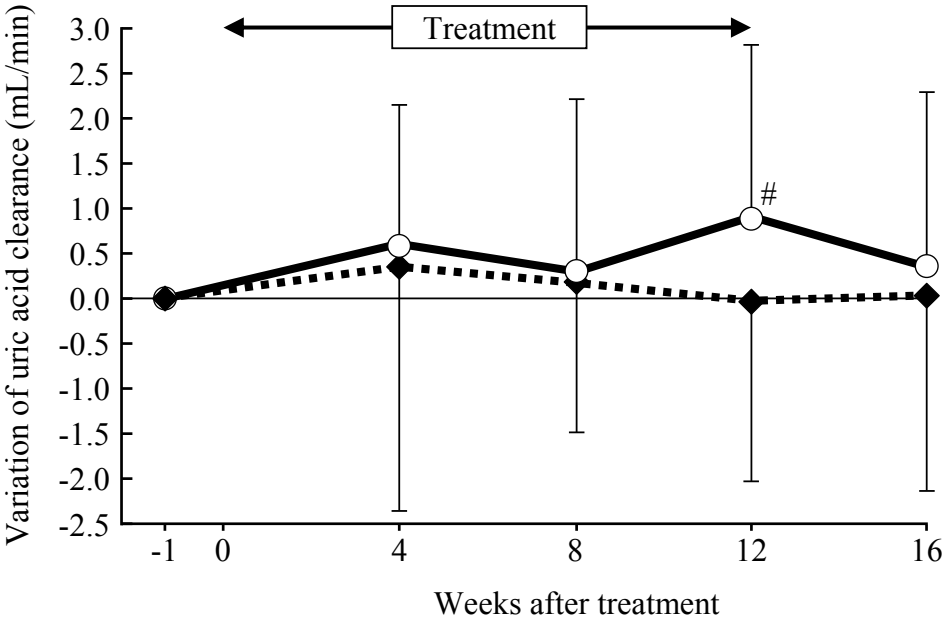


**Fig. 5-3. Time-course variation of uric acid clearance.**

Unfilled circles, treatment group (n=54); filled diamonds, placebo group (n=50). Each value is expressed as the mean ± SD. $^{\#}p<0.10$, compared with the placebo group using an analysis of covariance.

**Table 5-4. Results of the physical examination**

| Item | Group | 1 wk before treatment | 4 wks after treatment | 8 wks after treatment | 12 wks after treatment | 4 wks after termination |
|---|---|---|---|---|---|---|
| Weight (kg) | Test | 71.4±10.9 | 71.6±10.9 | 71.3±10.9 | 71.3±11.0 | 71.2±10.8 |
| | Placebo | 74.3±10.3 | 74.3±10.1 | 74.3±10.4 | 74.3±10.3 | 74.3±10.5 |
| BMI | Test | 24.6±3.7 | 24.6±3.7 | 24.5±3.7 | 24.5±3.7 | 24.5±3.6 |
| | Placebo | 25.6±3.4 | 25.6±3.3 | 25.6±3.4 | 25.6±3.4 | 25.6±3.5 |
| SBP (mm Hg) | Test | 127.2±15.1 | 128.7±13.4 | 127.1±13.5 | 127.4±12.4 | 127.3±13.5 |
| | Placebo | 127.8±12.4 | 126.9±10.0 | 125.7±12.1 | 125.2±10.7 | 127.1±11.6 |
| DBP (mm Hg) | Test | 79.1±11.1 | 78.9±11.3 | 76.2±11.7** | 76.2±12.0** | 77.3±12.3 |
| | Placebo | 80.6±10.6 | 80.9±9.1 | 78.1±10.1** | 76.7±9.6** | 79.0±9.6 |
| Pulse rate (beats/min) | Test | 73.1±10.2 | 73.6±11.3 | 72.5±10.9 | 72.3±10.5 | 73.6±11.1 |
| | Placebo | 75.5±9.5 | 73.5±9.9* | 73.6±9.9* | 74.1±11.4 | 72.9±10.4* |

SBP, systolic blood pressure; DBP, diastolic blood pressure.
Each value is expressed as the mean ± SD. Significant differences are as follows: $^{*}p<0.05$ and $^{**}p<0.01$, compared with the pre-treatment condition using the paired Student's *t*-test.

**Table 5-5-1. Results of the hematological analysis**

| Item | Normal range | Group | 1 wk before treatment | 4 wks after treatment | 8 wks after treatment | 12 wks after treatment | 4 wks after termination |
|---|---|---|---|---|---|---|---|
| WBC ($\times10^2/\mu L$) | M:39-98 F:39-91 | Test | 60.9±15.8 | 62.4±19.1 | 59.7±15.3 | 60.8±14.5 | 60.1±14.6 |
| | | Placebo | 59.8±12.7 | 59.7±13.9 | 60.1±13.0 | 59.7±14.4 | 60.4±14.0 |
| RBC ($\times10^4/\mu L$) | M:427-570 F:376-500 | Test | 493.3±39.3 | 489.2±33.7 | 493.9±36.0 | 487.6±38.1* | 491.1±36.8 |
| | | Placebo | 500.5±37.2 | 496.5±37.1 | 498.1±35.2 | 496.7±38.1 | 500.0±36.1 |
| Hb (g/dL) | M:13.5-17.6 F:11.3-15.2 | Test | 15.24±0.94 | 15.12±0.79 | 15.25±0.98 | 15.00±1.02* | 15.14±0.92 |
| | | Placebo | 15.16±0.99 | 15.03±0.92 | 15.08±0.88 | 15.05±0.99 | 15.12±0.94 |
| HMC (%) | M:39.8-51.8 F:33.4-44.9 | Test | 45.68±2.70 | 45.25±2.30 | 45.67±2.54 | 45.22±2.75* | 45.64±2.53 |
| | | Placebo | 45.68±2.62 | 45.37±2.52 | 45.47±2.43 | 45.72±2.77 | 45.95±2.61 |
| MCV (fL) | 85-100 | Test | 92.2±4.8 | 92.2±4.7 | 92.1±4.7 | 92.5±4.7 | 92.4±4.9 |
| | | Placebo | 92.2±2.9 | 92.0±2.7 | 92.1±2.8 | 94.0±4.1 | 94.3±5.3 |
| MCH (pg) | 28-34 | Test | 30.67±1.49 | 30.66±1.54 | 30.65±1.48 | 30.60±1.46 | 30.61±1.51 |
| | | Placebo | 30.58±1.13 | 30.62±1.28 | 30.43±1.13 | 30.45±1.04 | 30.40±1.06 |
| MCHC (%) | 31-35 | Test | 33.29±0.73 | 33.28±0.84 | 33.30±0.90 | 33.12±0.94 | 33.14±0.92 |
| | | Placebo | 33.19±1.12 | 33.27±0.99 | 33.11±0.80 | 32.44±1.28 | 32.32±1.48 |
| Platelets ($\times10^4/\mu L$) | 13-35 | Test | 24.70±4.51 | 24.57±5.16 | 25.13±4.82 | 25.20±4.66 | 24.76±4.66 |
| | | Placebo | 25.57±5.39 | 25.48±5.52 | 25.16±5.49 | 25.51±5.62 | 25.07±5.14 |

WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin level; HMC, hematocrit; M, male; F, female. Each value is expressed as the mean ± SD. Significant differences are as follows: $^*p<0.05$, compared with the pre-treatment condition using the paired Student's *t*-test.

**Table 5-5-2. Results of the serum biochemical analysis**

| Item | Normal range | Group | 1 wk before treatment | 4 wks after treatment | 8 wks after treatment | 12 wks after treatment | 4 wks after termination |
|---|---|---|---|---|---|---|---|
| FBS (mg/dL) | 70-109 | Test | 94.1±9.3 | 96.6±9.5* | 97.3±9.2** | 97.8±11.8** | 96.7±10.7** |
| | | Placebo | 96.5±8.6 | 94.7±7.9 | 95.1±7.8 | 96.6±9.7 | 95.0±9.6 |
| AST (IU/L) | 10-40 | Test | 23.5±7.3 | 22.2±7.2* | 22.9±7.7 | 22.6±10.0 | 23.8±12.9 |
| | | Placebo | 22.9±5.0 | 23.5±6.5 | 21.6±5.6 | 21.4±4.9 | 21.5±4.7 |
| ALT (IU/L) | 5-40 | Test | 29.2±21.7 | 27.6±20.4 | 28.7±21.2 | 28.1±22.2 | 30.3±26.8 |
| | | Placebo | 29.8±13.3 | 30.2±16.3 | 27.6±13.4* | 27.4±13.0* | 27.7±13.4 |
| ALP (IU/L) | 115-359 | Test | 200.4±46.4 | 199.7±53.3 | 207.0±54.4 | 204.8±50.7 | 207.0±60.1 |
| | | Placebo | 217.9±54.3 | 216.3±53.8 | 211.7±55.6* | 213.2±53.1 | 216.1±58.4 |
| γ-GTP (IU/L) | M:<70 F:<30 | Test | 41.8±27.4 | 41.0±29.0 | 40.7±27.7 | 40.3±24.2 | 43.3±36.5 |
| | | Placebo | 41.1±24.5 | 40.9±24.9 | 37.4±22.8** | 37.8±23.1* | 38.2±28.4 |
| LDH (IU/L) | 115-245 | Test | 170.8±25.3 | 167.8±25.4 | 169.6±27.1 | 169.5±28.0 | 168.8±26.3 |
| | | Placebo | 167.3±26.5 | 172.0±22.3 | 170.8±54.0 | 166.1±21.0 | 164.7±20.6 |
| Tbil (mg/dL) | 0.3-1.2 | Test | 0.73±0.26 | 0.71±0.27 | 0.71±0.34 | 0.75±0.35 | 0.74±0.32 |
| | | Placebo | 0.73±0.31 | 0.68±0.28 | 0.75±0.34 | 0.71±0.28 | 0.72±0.29 |
| TP (g/dL) | 6.7-8.3 | Test | 7.27±0.33 | 7.33±0.35 | 7.35±0.34 | 7.27±0.32 | 7.29±0.37 |
| | | Placebo | 7.37±0.36 | 7.34±0.32 | 7.32±0.36 | 7.30±0.34 | 7.30±0.34 |
| Alb (g/dL) | 4.0-5.0 | Test | 4.47±0.20 | 4.48±0.17 | 4.52±0.20* | 4.47±0.21 | 4.50±0.23 |
| | | Placebo | 4.53±0.24 | 4.49±0.24 | 4.47±0.23* | 4.49±0.24 | 4.49±0.26 |
| A/G ratio (%) | 1.2-2.0 | Test | 1.62±0.21 | 1.60±0.20 | 1.61±0.18 | 1.62±0.20 | 1.64±0.21 |
| | | Placebo | 1.61±0.21 | 1.59±0.18* | 1.59±0.19 | 1.62±0.22 | 1.62±0.19 |
| Tcho (mg/dL) | 150-219 | Test | 212.2±32.7 | 213.0±34.4 | 209.4±35.6 | 207.9±32.9 | 210.0±33.3 |
| | | Placebo | 215.5±35.8 | 212.0±36.4 | 212.8±35.4 | 212.1±37.8 | 212.7±34.5 |
| HDL-C (mg/dL) | M:40-86 F:40-96 | Test | 56.9±18.0 | 57.1±16.1 | 56.0±16.1 | 56.0±15.5 | 56.9±17.3 |
| | | Placebo | 53.5±15.6 | 52.8±16.1 | 53.1±15.7 | 52.5±15.3 | 53.2±15.5 |
| LDL-C (mg/dL) | 70-139 | Test | 128.7±33.4 | 131.9±34.8 | 128.1±35.1 | 127.9±33.5 | 129.2±31.0 |
| | | Placebo | 134.2±32.5 | 133.3±33.3 | 133.1±31.1 | 130.6±32.7 | 132.9±31.3 |
| TG (mg/dL) | 50-149 | Test | 148.7±102.5 | 134.0±83.4 | 135.7±112.7 | 134.7±75.9 | 125.9±60.9* |
| | | Placebo | 149.0±71.6 | 138.5±66.0 | 144.8±77.3 | 165.2±98.1 | 148.2±74.4 |
| BUN (mg/dL) | 8.0-22.0 | Test | 13.61±3.04 | 13.69±3.04 | 14.14±3.79 | 13.38±3.20 | 13.59±2.94 |
| | | Placebo | 13.29±2.85 | 13.80±3.10 | 13.32±2.31 | 13.01±2.28 | 13.35±2.33 |
| Cr (mg/dL) | 0.61-1.04 | Test | 0.84±0.10 | 0.83±0.10 | 0.84±0.10 | 0.83±0.10 | 0.84±0.11 |
| | | Placebo | 0.79±0.11 | 0.79±0.12 | 0.78±0.11 | 0.78±0.12 | 0.79±0.11 |
| UA (mg/dL) | M:3.7-7.0 F:2.5-7.0 | Test | 6.99±0.46 | 6.79±0.64 * | 7.02±0.73 | 6.78±0.62 ** | 6.96±0.68 |
| | | Placebo | 6.97±0.44 | 6.96±0.60 | 6.96±0.67 | 6.99±0.74 | 7.01±0.66 |
| Na (mEq/L) | 136-147 | Test | 141.0±1.7 | 141.1±1.8 | 141.0±1.6 | 140.9±1.9 | 141.3±1.7 |
| | | Placebo | 140.9±1.5 | 141.2±1.4 | 140.5±1.6 | 140.4±1.9 | 141.2±1.8 |
| K (mEq/L) | 3.6-5.0 | Test | 4.23±0.26 | 4.26±0.30 | 4.32±0.33 | 4.17±0.26 | 4.21±0.25 |
| | | Placebo | 4.20±0.27 | 4.29±0.37 | 4.44±1.15 | 4.34±1.13 | 4.12±0.24 |
| Cl (mEq/L) | 98-109 | Test | 103.9±1.9 | 103.8±2.0 | 103.7±1.7 | 104.1±1.9 | 104.1±2.0 |
| | | Placebo | 103.7±1.9 | 103.8±1.8 | 103.5±1.8 | 103.6±1.7 | 104.0±2.0 |
| Ca (mg/dL) | 8.7-10.1 | Test | 9.32±0.38 | 9.39±0.29 | 9.50±0.34* | 9.35±0.35 | 9.30±0.35 |
| | | Placebo | 9.33±0.34 | 9.33±0.31 | 9.39±0.26 | 9.38±0.26 | 9.36±0.34 |
| P (mg/dL) | 2.4-4.3 | Test | 3.41±0.45 | 3.40±0.53 | 3.50±0.56 | 3.31±0.43* | 3.41±0.42 |
| | | Placebo | 3.41±0.42 | 3.47±0.42 | 3.38±0.41 | 3.46±0.77 | 3.50±0.74 |

FBS, fasting blood sugar; Tbil, total bilirubin; TP, total protein; Alb, albumin; Tcho, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; TG, triglyceride level; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; M, male; F, female.

Each value is expressed as the mean ± SD. Significant differences are as follows: $^{*}p<0.05$, $^{**}p<0.01$, compared with the pre-treatment condition using the paired Student's *t*-test.

significant variations were found in such multiple items as FBS during the treatment period and post-experimental period, the physician in charge of the study judged that these changes were not outside the normal range of variation and did not cause any clinical problems.

*Urine test* The overall results are shown in Table 5-6. Certain samples exhibited abnormal changes: in the test group, one item with two subjects (two subjects were positive for urine protein), and in the placebo group, two items with three subjects (two subjects were positive for urine protein, and one subject was positive for urine occult blood). Although the measured values were found to exceed the normal range in certain subjects, the values from the examinations taken in advance and the examination of the values of the other items for these subjects, were adjudged to pose no clinical problems by the physician in charge of the study.

*Daily purine intake* Table 5-7 shows the change in the daily average purine intake that was calculated from the dietary surveys over 3 days prior to the examination. No significant changes were apparent in either group as compared to the pre-treatment levels.

*Daily alcohol intake* Changes in the daily average alcohol intake calculated from the meal diaries were as follows: test group, 1.1±9.2 g; placebo group, 3.2±11.5 g in weeks 1-4; test group, 0.3±9.7 g; placebo group, 1.7±8.8 g in weeks 5-8; test group, –0.1±8.7 g; placebo group, 0.5±8.3 g in weeks 9-12; and test group, 1.0±10.2 g; placebo group, 1.2±8.8 g in the post-experimental period. These figures did not reveal any significant changes.

**Table 5-6. Detection of proteins, glucose, and occult blood in the urine tests**

| Item | Group | | 1 wk before treatment | 4 wks after treatment | 8 wks after treatment | 12 wks after treatment | 4 wks after termination |
|---|---|---|---|---|---|---|---|
| Protein | Test | – | 55 | 53 | 55 | 51 | 53 |
| | | ± | 1 | | | 2 | |
| | | + | | 3 | | 2 | 2 |
| | Placebo | – | 53 | 54 | 51 | 52 | 52 |
| | | ± | | | 2 | | 2 |
| | | + | 2 | 1 | 1 | 3 | 1 |
| Glucose | Test | – | 56 | 56 | 55 | 55 | 55 |
| | | ± | | | | | |
| | | + | | | | | |
| | Placebo | – | 55 | 55 | 54 | 55 | 55 |
| | | ± | | | | | |
| | | + | | | | | |
| Occult blood | Test | – | 54 | 52 | 53 | 54 | 54 |
| | | ± | 2 | 4 | 2 | 1 | 1 |
| | | + | | | | | |
| | Placebo | – | 52 | 53 | 50 | 53 | 50 |
| | | ± | | | 1 | | 1 |
| | | + | 2 | 2 | 2 | 2 | 2 |
| | | ++ | 1 | | | | 2 |
| | | +++ | | | 1 | | |

Numerals represent the number of cases showing the respective symptoms.
Proteins, glucose, and occult blood were graded as negative (–), weakly (±), mildly (+), intermediately (++), or severely (+++) positive.

**Table 5-7. Daily purine intake during the study period**

| Item | Group | 1 wk before treatment | 4 wks after treatment | 8 wks after treatment | 12 wks after treatment | 4 wks after termination |
|---|---|---|---|---|---|---|
| Purine (mg/day) | Test | 319.0±78.6 | 313.9±109.4 | 299.4±84.7 | 301.0±70.3 | 294.6±96.9 |
| | Placebo | 323.0±110.1 | 306.3±98.5 | 314.6±98.1 | 299.0±86.1 | 319.7±105.3 |

The daily purine intake was estimated on the basis of the 3-day dietary surveys taken before the clinical examinations. Each value is expressed as the mean ± SD.

## 5.4 Discussion

FBEP, a primary component of the test food in the present study, had been found to significantly decrease the serum UA level in an open study using male Japanese subjects with a serum UA level in the range of 6.0-7.9 mg/dL.[28] Based on this result, we carried out a double-blind, parallel-group study of Japanese men and women with slightly elevated levels of serum UA to statistically confirm the effect of FBEP on decreasing the serum UA level (after having eliminated environmental factors and the placebo effects) and also to examine the safety of FBEP. We found that the FBEP-treated group exhibited a significantly lower serum UA level in week 12 of the treatment period than the placebo group. The within-group comparison revealed that the test group exhibited significantly lower serum UA levels in week 4 and 12 of the treatment period than the pre-treatment levels, but that the levels in week 8 of the treatment period returned to the pre-treatment levels. Since the placebo group demonstrated little change in the serum UA level throughout the examination period, we examined the factors influencing the serum level of UA of the test group in week 8 of treatment. The purine and alcohol intake are closely related to serum UA, but no elevation of the intake of purine or alcohol by the test group was apparent in week 8 of treatment. The renal function is also closely related to serum UA, but no alteration in serum creatinine or blood urea nitrogen was apparent. In the case of the test group, the standard deviation of 0.73 in serum UA level in week 8 of treatment shows slight elevation, as compared to 0.64 in week 4 of treatment and to 0.62 in week 12 of

treatment, indicating a wide variation of the measured values in week 8 of the treatment period.

On the other hand, an evaluation of the UA clearance that was carried out to examine a possible mechanism of action, and the test group showed a slightly higher level in week 12 of treatment than the placebo group. Based on this result, it is suggested that the action of FBEP in reducing serum UA can possibly be attributed to the accelerated excretion of UA. Among the 54 subjects in the test group, the serum UA level in 10 subjects with a normal range of UA clearance was 6.94 mg/dL prior to treatment, changing to 6.88 mg/dL in week 4 of treatment, to 7.25 mg/dL in week 8 of treatment, and to 6.95 mg/dL in week 12 of treatment, although these changes were not significant throughout the period of intake. The influence of FBEP on the serum UA level in the subjects with an excessive production of UA is considered to have been low.

As characteristic components contained in FBEP, the secondary metabolites of *Aspergillus* species, which possesses antioxidant activity, have been reported.[99] On the other hand, the intake of vitamin C, a water-soluble antioxidant, reduces the serum UA level.[100] Moreover, higher vitamin C intake has been independently associated with a lower risk of gout.[101] An increase in glomerular filtration and/or competition for renal reabsorption are considered as the mechanism by which vitamin C reduded serum UA. The mechanism by which FBEP reduced serum UA may have inhibited the renal reabsorption of UA by an ingredient like the vitamin C reabsorbed *via* anion-exchange tansport at proximal tubules.

Furthermore, it has been reported that consumption of cherries including polyphenols in a healthy women increased urinary UA excretion, and decreased plasma UA.[102)]

On the other hand, it has been reported that an intake of the milk and soy origin protein increased urinary UA excretion and clearance.[78)] Furthermore, it has been reported that urinary UA excretion increased by the intravenous administration of amino acids.[103)] That is, it is necessary to examine the influence of anionic peptides contained in FBEP on the UA excretion.

The detailed mechanism for the molecular action of FBEP on the accelerated excretion of UA remains for future study. However, we have been examining the orotic acid-induced inhibitory effect of FBE on fatty liver in rats.[3,4)] It has been reported that the level of human URAT 1 was greatly reduced by a high level (1 mM) of orotic acid,[23)] and that URAT 1 was responsible for transporting orotic acid across cell membranes, this transport being strongly blocked by benzbromarone which is a UA excretion-promoting drug.[24)] In addition, human organic anion transporter (hOAT) 1 and hOAT 3 may be transporting some UA from the lumen of the blood vessels to renal tubular cells.[104,105)] It is also known that there is gender difference in the serum UA level,[106)] for which UA clearance increased by estrogen has been reported to be a cause.[107)] In other words, the UA transporter may be controlled by sex hormones. We now plan to carry out a further study identifying which constituent in the crude composition of FBEP was involved in mediating the serum UA-lowering effect, while paying attention to the organic anion

transporter.

The safety of FBEP during 12 weeks of continuous intake, at 2 g/day, was confirmed by the findings that there was little change to cause clinical concern in the examined parameters (Table 5-4, 5-5, 5-6), and that there were no symptomatic manifestations. FBEP obtained from the combination of barely and the brewing microorganism, whose safety has been confirmed through the long years of dietary experience, is expected to be utilized as a side-effect-free, safe functional food material.

In conclusion, the results of the present study indicate that FBEP obtained by fermenting barley with fungus and yeast had the effect of significantly reducing the serum UA level in subjects with slightly elevated serum UA or mild hyperuricemia.

## 5.5 Summary

The uric acid (UA)-lowering effect and safety of a fermented barley extract P (FBEP) prepared from barley-*shochu* distillery by-products were investigated in a randomized, placebo-controlled, parallel-group, double-blinded study. A total of 111 subjects with serum UA levels of 6.0-7.9 mg/dL were provided with either a drink containing 2 g/day of FBEP (test group) or a placebo drink (placebo group). After 12 weeks, the serum UA levels changed by –0.21±0.56 mg/dL in the test group, showing a significant decrease in comparison to those of the placebo group (+0.02±0.54 mg/dL). Additionally, the UA clearance in the test group showed a tendency to increase after 12 weeks more than in the placebo group ($p$=0.054). No

abnormalities in the physical and clinical tests were observed, and no adverse diagnostic findings were attributed to the intake of the test meal. These results demonstrated the benefits and safety of the FBEP treatment to subjects with slightly high serum UA or mild hyperuricemia.

# Chapter 6
# Conclusion

The *honkaku-shochu* industry, as a whole, produces about 800,000 tons of SDB annually. The amount that is effectively utilized as fertilizer, feed, and food materials is about 30% of the total volume, with much of the remainder treated as industrial waste. Economic feasibility is important for the continuous and stable utilization of by-products. Such a viewpoint has promoted the need to find new uses for SDB and enhance its utilization in advanced technologies. Hence, in these studies, animal experiments and clinical applications were conducted to evaluate the physiological functions of SDB, an unused resource, in order to utilize SDB for advanced technologies and for use as a functional food material with benefits to humans.

In chapter 2, the effects of dietary supplementation with FBE on lipid metabolism and antioxidant status in mice are presented. In Exp. 1, male 5-month-old mice were fed either a control diet or an experimental diet containing 10% barley, fermented barley fiber (striated part of barley grain) or FBE for 3 months. In Exp. 2, male 5-month-old mice were fed either a control diet or experimental diet containing FBE or FBEP for 3 months. TBARS concentrations in both plasma and liver were significantly lower in the FBE and FBEP groups than the control group. Erythrocyte GPx activity was significantly higher in the FBEP group than in the other groups. Liver GSH content was significantly higher in the FBEP group than in the

control group. These results suggested that FBEP, prepared from the water-soluble fraction of barley-SDB, significantly reduces oxidative stress in the body.

In chapter 3, the effects of single and combined administration of FBEP and GABA on the development of AD-like skin lesions in NC/Nga mice are presented. Single administration of FBEP and GABA dose-dependently reduced the development of AD-like skin lesions in NC/Nga mice. GABA reduced the development of AD-like skin lesions by suppressing serum IgE and splenocyte IL-4 production, while FBEP reduced skin lesions without affecting the IgE and cytokine production. However, in mice with induced AD-like skin lesions, combined administration of FBEP and GABA decreased serum IgE levels and splenic cell IL-4 production, and increased splenic cell IFN-γ production. These results suggest that combined administration of FBEP and GABA alleviated AD-like skin lesions in the NC/Nga mice by adjusting the Th1/Th2 balance to a Th1-predominant immune response.

In chapter 4, the effects of a FBEP on UA metabolism in three hyperuricemia rat models are presented. First, the influence of FBEP on UA biosynthesis was examined using rats hypodermically administrated oxonic acid. Then, the influence of FBEP on dietary purine absorption was examined using rats fed a high dose of inosinic acid. Finally, the influence of FBEP on UA excretion was examined using adenine-administered rats. From the first two models, FBEP was found to be rarely, or not at all, involved in the biosynthesis of UA or absorption of nucleic acid. On the other hand, in the rats

loaded with adenine, it was shown that the urinary UA of the high-dose FBEP group (4,000 mg/kg/day) was significantly elevated by 27% compared to the control group. In addition, the UA clearance value in the high-dose FBEP group was increased 2-fold compared to the control group. These results suggest that FBEP reduces serum UA by increasing urinary excretion of UA.

In chapter 5, the UA-lowering effect and safety of a FBEP are presented for a randomized, placebo-controlled, parallel-group, double-blinded study. The subjects in this clinical study consisted of 111 Japanese men and women with serum UA levels of 6.0-7.9 mg/dL, who were provided with either a drink containing 2 g/day of FBEP (test group) or a placebo drink (placebo group). After 12 weeks, the serum UA levels changed by –0.21±0.56 mg/dL in the test group, showing a significant decrease in comparison to those of the placebo group (+0.02±0.54 mg/dL). Additionally, the UA clearance in the test group showed a tendency to increase after 12 weeks more than in the placebo group ($p$=0.054). No abnormalities in the physical and clinical tests were observed, and no adverse diagnostic findings were attributed to the intake of the test meal. These results demonstrated the benefits and safety of the FBEP treatment to subjects with slightly high serum UA or mild hyperuricemia.

Taken together, these results were successfully applied to the development of SDB as a functional food material that exhibits antioxidative and anti-inflammatory activities in animals and improves UA metabolism in humans. Part of these study results was used to develop beverages containing FBEP, and preparations to

supply FBEP to food producers are in progress. This study elucidated the *in vivo* physiological functions of FBEP to convert SDB, previously considered a waste product, into a functional food material with a high added value. Further scientific data accumulation and development of efficient separation and purification techniques as well as mass-production methods for the target functional substances are expected to contribute not only to building sustainable resource recycling processes, but also to future creation of new industries.

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# Publications

(1) Effects of Fermented Barley Extract on Antioxidant Status in Mice.
Hideki Hokazono, Toshiro Omori, Hiramitsu Suzuki and Kazuhisa Ono
Food Science and Technology Research, **15** (6), 599-604 (2009).

(2) Effects of Single and Combined Administration of Fermented Barley Extract and γ-Aminobutyric Acid on the Development of Atopic Dermatitis in NC/Nga Mice.
Hideki Hokazono, Toshiro Omori and Kazuhisa Ono
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(3) Anti-hyperuricemic Effect of Fermented Barley Extract is Associated with Increased Urinary Uric Acid Excretion.
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