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Biological Activity of Bamboo Salt

Hye-Young Shin[1,2], Ho-Jeong Na[1,2], Phil-Dong Moon[1,2], Sang-Wan Seo[2], Tae-Yong Shin[4], Seoung-Heon Hong[2], Ki-Nam Lee[5], Rae-Kil Park[3] and Hyung-Min Kim[1†]

[1]*Department of Pharmacology, College of Oriental Medicine, Kyung Hee University, Seoul 130-701, Korea*
[2]*College of Pharmacy,* [3]*VCRC of Wonkwang University, Jeonbuk 570-749, Korea*
[4]*College of Pharmacy, Woosuk University, Jeonbuk 565-701, Korea*
[5]*Professional Graduate School, Wonkwang University, Jeonbuk 570-749, Korea*

ABSTRACT

Bamboo salt has been used for the purpose of prevention and treatment of various diseases in Korea. Present study was carried out to ascertain the effects of purple bamboo salt upon anti-allergic effect, anti-inflammatory activity and immune-enhance effect as well. Purple bamboo salt significantly inhibited the ear swelling response and histamine release induced by compound 48/80 in mice and rat peritoneal mast cells. Purple bamboo salt (0.01~1 g/kg) also dose-dependently inhibited the passive cutaneous anaphylaxis by oral administration. Purple bamboo salt (1 mg/mL) inhibited phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 secretion, by 67.04±0.08%, 68.01±1.85%, 69.48±0.54%, respectively. In addition, purple bamboo salt inhibited the expression of TNF-α mRNA in HMC-1 cells. Finally, we investigated the effect of purple bamboo salt in the forced swimming test (FST) and the change of purple bamboo salt-mediated cytokine production from MOLT-4 cells. At the 7th, immobility time was significantly decreased in the purple bamboo salt-administration group (35.4± 5.9 s for 1 g/kg) in comparison with the control group (93.2±15.4 s). After FST, the content of glucose in the blood serum was increased and the levels of blood urea nitrogen, lactic dehydrogenase was decreased in purple bamboo salt-administration group. However, it had no effect on the elevation of CK and TP level. Purple bamboo salt (1 mg/mL) significantly increased the interferon (IFN)-γ and IL-2 level compared with media control (about 3.7-fold for IFN-γ, about 3.5-fold for IL-2, $p<0.05$) but did not affect the IL-4.

Key words: purple bamboo salt, immediate-type allergic reactions, anti-inflammatory activity, immune-enhance effect

INTRODUCTION

Purple bamboo salt is a specially processed salt according to the traditional recipe using normal salt and bamboo in Korea. It is known to have various therapeutic effects on diseases such as inflammations, viral disease, diabetes, circulation organ disorder and cancer etc (1-4). However, its possible pharmacological mechanism has not been investigated clearly. This report describes an effect of purple bamboo salt on mast cell-mediated immediate-type allergic reactions, inflammatory activity and also immune-enhance effect.

Mast cells are key regulators in allergy and inflammation, and release histamine, cytokine and other pro-inflammatory mediators (5). In general, immediate hypersensitivity, which involves urticaria, allergic rhinitis and asthma, is mediated by various chemical mediators release from mast cells (5). Degranulation of mast cells is caused by non-immunologic secretagogues like substance P, compound 48/80, extracellular ATP and so on, which result in rapid and marked histamine release (6-9). Studies on the compound 48/80-induced mast cell degranulation and ear swelling response have been continuously performed on these theoretical bases by our group (10-12). Among the preformed and newly synthesized inflammatory substances released on de-

†Corresponding author. E-mail: hmkim@khu.ac.kr
Phone: 02-961-9448, Fax: 02-968-1085

granulation of mast cells, histamine remains the best characterize and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (13). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen (14-16). The anti-IgE antibody has been established to induce passive cutaneous anaphylaxis (PCA) reactions as a typical in vivo model for immediate hypersensitivity in allergic cutaneous reactions (17). Although mast cells also store small amounts of cytokines in their granules (18), these cells dramatically increase the production of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 and other cytokines after stimulated with phorbol 12-myristate 13-acetate plus calcium ionophore A23187. The finding that activated mast cells are also a source of several cytokines suggests an additional role of mast cells in late-phase reactions and other persistent inflammatory processes (19).

Next, the forced swimming test (FST) is commonly used to evaluate anti-depressants, and many anti-depressants show the anti-immobility effects (20,21). It has been reported that FST exposure produces alterations in both cellular and noncellular immunity (22). FST exposure caused a reduction in the percentage of lymphocytes and an increase in the percentage of neutrophils in the peripheral blood and there was a significant but transient suppression of both PHA (phytohemagglutinin) and Con A-induced lymphocyte proliferation following FST exposure (23,24).

FST is used to examine whether a certain agents has anti-fatigue effect and used for endurance test (25-29). The blood urea nitrogen (BUN), creatine kinase (CK), lactic dehydrogenase (LDH), glucose (Glc) and total protein (TP) are blood biochemical parameters related to fatigue. As is commonly known, Glc level is decreased immediately after exercise. The BUN test is a routine test used primarily to evaluate renal function. Serum LDH and CK is known to be accurate indicators of muscle damage (30,31). LDH catalyzes the interconversion of pyruvate and lactate. Most of the CK in the body normally exists in muscle, a rise in the amount of CK in the blood indicates that muscle damage has occurred, or is occurring. TP is a rough measure of serum protein. Enzymes, some hormones, hemoglobin, LDL, fibrinogen, immunoglobulins are some examples of proteins (32).

T-cells play a crucial role in immune functions as they act both as effectors (cytotoxic T-cells, Tc cells) and regulators (helper and suppressor T-cells, Th and Ts cells). The existence of T helper type Th1/Th2 subsets in Th lymphocytes that differ in their cytokine secretion patterns and effector functions provides a framework for understanding normal and pathological immune responses (33,34). Induction of Th1 immune responses plays a critical role in protecting against various intracellular micro-organisms and tumors, and also in reversing Th2 cell-facilitating diseases such as allergic inflammation (35-37). The Th1/Th2 classification has been useful in relating the overall patterns of cytokine production to clinical outcomes in a variety of pathological states (38).

In the present study we examined the effects of purple bamboo salt in the anti-allergic effect, anti-inflammatory activity and immune-enhancing effect.

MATERIALS AND METHODS

Materials

Compound 48/80, anti-DNP IgE, metrizamide, o-phthaldialdehyde (OPA), and Evans blue and Avidin-peroxidase and 2'-AZINO-bis (3-ethylbenzithiazoline-6-sulfonic acid) tablets substrate were purchased from Sigma chemical Co. (St. Louis, MO, USA). The α-minimal essential medium was purchased from Flow Laboratories (Irvine, UK). RPMI 1640, ampicillin, streptomycin and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum was purchased from Life Sciences (Grand Island, NY, USA). Anti-human TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 biotinylated anti-human TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 and recombinant (r) human TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 were purchased from R&D Systems (Minneapolis, MN, USA).

Animals

The original stock of ICR mice and Wistar rat was purchased from the Dae-Han Experimental Animal Center (Seoul, Korea), and the animals were maintained in the College of Pharmacy, Wonkwang University. The rats were housed five to ten per cage in a laminar air-flow room maintained at a temperature of 22±1°C

and relative humidity of 55±10% throughout the study. ICR mouse were killed in accordance with National Institutes of Health animal care and use guidelines.

Preparation of purple bamboo salt

Purple bamboo salt (Sambou purple bamboo salt) was provided by Tae Sung Food Inc. (Jeonbuk, South Korea). It was processed by special technique (9 times processing at very high temperature with bay salt, bamboo, pine tree firewood, pine resin, and yellow earth etc.) of Hyo-San Snim (intangible cultural properties No 23 of purple bamboo salt manufacture part and the former head priest of Buddhist Gae-Am Temple, Jeonbuk, South Korea). Powdered purple bamboo salt and NaCl were melted in distilled water to reach appropriate concentrations, filtered through 0.45 μm filter, and kept at 4°C.

Ear Swelling Response

Compound 48/80 was freshly dissolved in saline and injected intradermally into the dorsal aspect of a mouse ear using a microsyringe with a 28-gauge hypodermic needle. Ear thickness was measured with a digimatic micrometer (Mitutoyo, Japan) under mild anesthesia. Ear swelling response represented an increment in thickness above baseline control values. Ear swelling response was determined 40 min after compound 48/80 or vehicle injection. Purple bamboo salt was administered orally 1 h before the compound 48/80-injection (100 μg/site). The values obtained would appear to represent the effect of compound 48/80 rather than the effect of the vehicle injection (physical swelling), since the ear-swelling response evoked by physiologic saline returned to almost baseline thickness within 40 min.

Preparation of Rat Peritoneal Mast Cells (RPMC)

RPMC were isolated as previously described (39). In brief, rats were anesthetized by ether, and injected with 20 mL of Tyrode buffer B (NaCl, glucose, $NaHCO_3$, KCL, NaH_2PO_4) containing 0.1% gelatin (Sigma Chemical. Co.) into the peritoneal cavity; the abdomen was gently massaged for about 90 sec. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by Pasteur pipette. Then the peritoneal cells were sedimented at 150×g for 10 min at room temperature and resuspened in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells (i.e. macrophages and small lymphocytes) according to the method described by Yurt et al. (40). In brief, peritoneal cells suspended in 1 mL of Tyrode buffer B were layered onto 2 mL of 0.25 g/mL metrizamide (density 1.120 g/mL; Sigma) and centrifuged at room temperature for 15 min at 400×g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 mL of Tyrode buffer A containing calcium. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake.

Histamine Release

Purified RPMC were resuspended in Tyrode buffer A containing calcium for the treatment with compound 48/80. RPMC suspensions (2×10^5 cells/mL) were preincubated for 10 min at 37°C before the addition of compound 48/80 for stablization. The cells were preincubated with the purple bamboo salt for 20 min, and then incubated for 15 min with compound 48/80 (6 μg /mL). The reaction was stopped in ice. The cells were separated from the released histamine by centrifugation at 400×g for 5 min at 4°C. Residual histamine in the cells was released by disrupting the cells with perchloric acid and centrifugation at 400×g for 5 min at 4°C.

The inhibition percentage of histamine release was calculated using the following equation:

% Inhibition = {(Histamine release without purple bamboo salt-Histamine release with purple bamboo salt) / Histamine release without purple bamboo salt}×100

PCA Reaction

IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 24 h later with an injection of DNP-HSA into the mice tail vein. The DNP-HSA was diluted in phosphate-buffered saline (PBS). The mice was injected intradermally with 100 ng of anti-DNP IgE into each of 3 dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. 24 h later, each mice received an injection of 200 μL of the 1 : 1 mixture of 1 mg/mL

DNP-HSA in PBS and 4% Evans blue via the tail vein. One hour before this injection, purple bamboo salt was administered orally. And the mice were sacrificed 40 min after the i.v.-challenge. The dorsal skin of the mouse was removed for measurement of the pigment area. The amount of dye was then determined colorimetrically after extraction with 0.5 mL of 1.0 N KOH and 4.5 mL of a mixture of acetone and phosphoric acid (with the ratio of 5 : 13), based on the method of Katayama et al. (41). The absorbent intensity of the extraction was measured at 620 nm in a spectrofluorometer, and the amount of dye was calculated with the Evans blue measuring-line.

Cell culture

Human leukemic cell line HMC-1 cells were grown in IMDM medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 10^{-5} M monothioglycerol and 10% heat-inactivated FBS at 37°C in 5% CO_2 and 95% humidity. Cells were treated purple bamboo salt for 30 min prior to stimulation with 20 nM PMA plus 1 μM A23187 and incubated at 37°C for 8 h. T cell line MOLT-4 cells were grown in RPMI 1640 medium (Gibco BRL, USA) supplemented with 10% fetal bovine serum (JRH BIOSCIENCE, USA), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in the presence of 5% CO_2.

MTT assay

Cell viability was determined by the MTT assay. Briefly, 500 μL of HMC-1 cells suspension (2.5×10^4 cells) was cultured in 4-well plates for 24 h after treatment by each concentration of purple bamboo salt. 20 μL of MTT solution (5 mg/mL) was added and the cells were incubated at 37°C for an additional 4 h. After washing the supernatant out, the insoluble formazan product was dissolved in DMSO. Then, optical density of 96-well culture plates was measured using an enzyme-linked immunosorbent assay (ELISA) reader at 540 nm. The optical density of formazan formed in untreated control cells was taken as 100% of viability.

Cytokines assay

Sandwich enzyme-linked immunosorbent assay (ELISA) for TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 was carried out in duplicate in 96-well ELISA plates (Nunc, Denmark) coated with each of 100 μL aliquots of anti-human TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 monoclonal antibodies at 1.0 μg/mL in PBS at pH 7.4 and was incubated overnight at 4°C. The plates were washed in PBS containing 0.05% Teeen-20 (Sigma, St. Lousis, MO, USA) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 for 1 h. After additional washes, sample or TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 standards were added and incubated at 37°C for 2 h. After 2 h incubation at 37°C, the wells were washed and then each of 0.2 g/mL of biotinylated anti-human TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 were added and again incubated at 37°C for 2 h. After washing the wells, avidin-peroxidase was added and plates were incubated for 20 min at 37°C. Wells were again washed and ABTS substrate was added. Color development was measured at 405 nm using an automated microplate ELISA reader. A standard curve was run on each assay plate using recombinant TNF-α/IL-1β/IL-6 and IFN-γ/IL-2/IL-4 in serial dilutions.

RT-PCR

Total RNA was isolated from HMC-1 cells with Easy-blue according to the manufacturer's instruction, PCR was performed with following primers for TNF-α (5'CGG GAC GTG GAG CTG GCC GAG GAG3'; 5'CAC CAG CTG GTT ATC TCT CAG CTC 3'). The actin (5' GTG GGG CGC CCC AGG CAC CA3'; 5'GTC CTT AAT GTC ACG CAC GAT TTC3') was used to verify that equal amounts of RNA were used for reverse transcription and PCR amplification from different experimental conditions. The annealing temperature was 60°C for TNF-α and actin. Amplified fragment sizes for TNF-α and actin were 355 bp and 661 bp, respectively. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

FST

During the 6 min of the FST, the duration of immobility was measured as previously described by Porsolt and co-workers (20). The apparatus consisted of two Plexiglas cylinders (height: 25 cm, diameter: 10 cm) placed side by side in a Makrolon cage filled with water (10 cm height) at 23~25°C. Two mice were tested simultaneously for a 6-min period inside vertical Plex-

iglas cylinders; a nontransparent screen placed between the two cylinders prevented mice from seeing each other. The total duration of immobility, after a delay of 2 min, was measured during a period of 4 min. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

Preparation and ingredient analysis of blood serum

Mice were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). After anesthetization, bloods were withdrawn from heart of forced swimming-treated mice into syringes. Then, serum was prepared by centrifugation at 3000 rpm at 4°C for 10 min. Contents of Glc, LDH, BUN, CK and TP were determined by the autoanalyzer (Hitachi 747, Hitachi, Japan).

Western blot analysis

Cell extracts were prepared by detergent lysis procedure. Cells (5×10^6 cells) were scraped, washed once with PBS, resuspended in lysis buffer. Samples were vortexed for lysis for a few seconds every 15 min at 4°C for 1 h and centrifuged at 15 000×g for 5 min 4°C. Samples were heated at 95°C for 5 min, and briefly cooled on ice. Following the centrifugation at 15 000×g for 5 min, 50 μL aliquots were resolved by 12% SDS-PAGE. Resolved proteins were transferred overnight to nitrocellulose membrane in 25 mM Tris, pH 8.5, 0.2 mM glycerin, 20% methanol at 25 V. Blots were blocked for at least 2 h with 1×TBST containing 10% nonfat dry milk. Protein levels were analyzed essentially according to the manufacturer's instructions.

Statistical analysis

The results were expressed as mean±SEM for the number of experiments. Statistical significance was compared between each treated group and control by the Student's *t*-test. Results with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Anti-allergic effect

To examine the anti-allergic effect, we carry out the compound 48/80-induced ear swelling response and anti-DNP IgE-induced PCA reactions. Also, we investigate the compound 48/80 induced histamine release from RPMCs. We chose concentration of 100 μg/site for compound 48/80 induced optimal ear-swelling response in this experiment. As shown in Table 1, when mice were pretreated with purple bamboo salt for 1 h, the ear swelling response derived from compound 48/80 was reduced. We next examined the effect of purple bamboo salt on compound 48/80-induced histamine release from RPMC. Purple bamboo salt dose-dependently inhibited the compound 48/80-induced histamine release (Fig. 1). The report that compound 48/80 increased the per-

Table 1. Effect of purple bamboo salt on compound 48/80-induced ear swelling response in mice[1)]

Purple bamboo salt addition (g/kg)[2)]	Thickness (mm)	Inhibition (%)[3)]
None	0.247±0.004	-
0.01	0.170±0.008*	29.96
0.1	0.957±0.003*	60.73
1	0.120±0.002*	51.32

[1)]20 μL of compound 48/80 (100 μg/site) were applied intradermally.
[2)]The mice were orally administered with the indicated concentration of purple bamboo salt for 1 h prior to the compound 48/80 application.
[3)]Each datum represents the mean±SEM of three independent experiments.
*$p<0.05$: significantly different from the saline value.

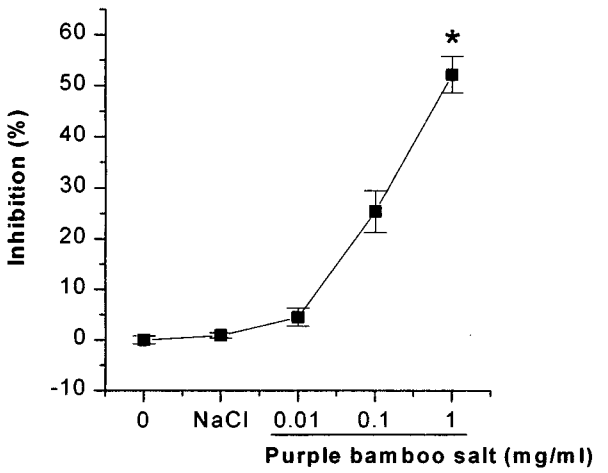

Fig. 1. Effects of purple bamboo salt on compound 48/80-induced histamine release from RPMCs.
RPMCs (2×10^5 cells/mL) were preincubated with drug at 37°C for 10 min prior to incubation with compound 48/80. Each datum is presented as the SEM of three independent experiments. *$p<0.05$: significantly different from the control value.

meability of the lipid bilayer membrane by causing a perturbation of the membrane indicates that the membrane permeability increase may be an essential trigger for the release of mediators from mast cells (42). So, it is possible to suppose that purple bamboo salt might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80. Another way to test skin allergic reaction is to induce PCA (18). Local injection of anti-DNP IgE followed by an intravenous antigenic challenge has been performed. Anti-DNP IgE was injected into dorsal skin sites. After 24 h, all animals were injected i.v. with DNP-HSA containing Evans blue dye. The cutaneous anaphylactic reaction was best visualized by the extravasation of dye. When purple bamboo salt (0.01~1 g/kg) was orally administered to the mice, PCA reaction was inhibited dose-dependently (Table 2).

The mechanism of the protection against anti-DNP IgE, while not clear at present, may be suggested only in some particular conditions. It is conceivable that purple bamboo salt inhibits the initial phase of immediate type allergic reactions, probably through interference with the degranulation system. In conclusion, the results obtained in the present study provide evidence that purple bamboo salt inhibited the immediate-type allergic reactions in vivo and in vitro in a murine model.

Table 2. Effect of purple bamboo salt on 48 h PCA in mice

Purple bamboo salt addition (g/kg)[1)]	Amount of dye (μg/site)[2)]	Inhibition (%)
None (distilled water)	0.7598±0.11256	-
0.01	0.6942±0.1465	11.1
0.1	0.5749±0.08565	24.3
1	0.3650±0.01383*	51.9*

[1)]Purple bamboo salt dissolved with distilled water was orally administered to mice 1 h before the challenge.
[2)]Each value indicates mean±SEM.
*$p<0.05$: significantly different from the saline value.

Anti-inflammatory activity

We examined the inhibitory effect of purple bamboo salt on the PMA plus A23187-stimulated secretion of TNF-α, IL-1β, and IL-6 from HMC-1 cells. Culture supernatants were assayed for each cytokines levels by ELISA method. As shown in Table 3, purple bamboo salt inhibited the secretion of TNF-α, IL-1β, and IL-6 in PMA plus A23187-stimulated HMC-1 cells. NaCl also inhibited TNF-α and IL-6 but NaCl exhibited markedly lower effect than the purple bamboo salt. Also, both purple bamboo salt and NaCl did not directly affect HMC-1 cell cytotoxicity (Fig. 2). We examined the inhibitory effect of purple bamboo salt on the expression of TNF-α mRNA in PMA plus A23187-stimulated HMC-1 cells through RT-PCR. Purple bamboo salt inhibited the TNF-α mRNA expression induced by PMA plus A23187 (Fig. 3). Chronic inflammation, as evidenced by increased levels of proinflammatory cytokines and C-reactive protein, is a common feature in dialysis patients and is associated with an increased cardiovascular morbidity and mortality (43). Therefore our results suggest that purple bamboo salt importantly contributes to the prevention or treatment of inflammatory diseases.

Immune-enhancing effect

We have examined the endurance effect of purple bamboo salt in the FST and the change of purple bamboo salt-mediated cytokine production. Purple bamboo salt (1 g/kg) was orally administered to mice for 7 days. We measured the immobility time at the 2, 7days after the administration of saline, NaCl, purple bamboo salt. As a result of test, the immobility time was decreased in the purple bamboo salt-administrated group

Table 3. Effect of purple bamboo salt on the secretion of PMA plus A23187-stimulated TNF-α, IL-1β, IL-6 in HMC-1 cells

Treatment	Concentration (mg/mL)	Inhibition (%)		
		TNF-α	IL-1β	IL-6
Saline	-			-
Purple bamboo salt	0.01	26.65±1.35	7.09±0.14	7.13±3.45
	0.1	46.12±0.15	59.03±2.09*	45.03±1.32*
	1	67.04±0.08*	68.01±1.85*	69.48±0.54*
NaCl	1	24.42±1.58*	-	27.57±1.82

PMA plus A23187-stimulated HMC-1 cells (3×10^5) were incubated for 6 h in the absence or presence of purple bamboo salt or NaCl. TNF-α, IL-1β, IL-6 secreted into the medium are presented as the mean±SEM of three independent experiments.
*$p<0.05$: significantly different from the saline value.

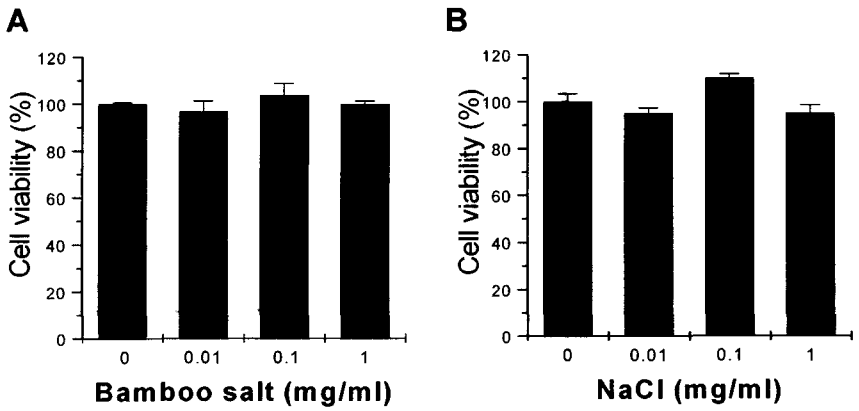

Fig. 2. Effect of purple bamboo salt on the cell viability in HMC-1 cells.
Cell viability was evaluated by MTT assay 24 h after purple bamboo salt and NaCl treatment (0.01~1 mg/mL) in HMC-1 cells. The percentage of viable cells was over 95% in each condition. Data represent the mean±SEM of three independent experiments.

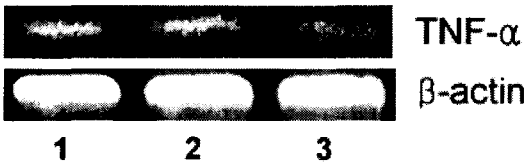

Fig. 3. Effect of purple bamboo salt on the expression of TNF-α mRNA in HMC-1 cells by RT-PCR.
Total RNA was isolated from HMC-1 cells. HMC-1 cells were pretreated with purple bamboo salt for 30 min prior to PMA plus A23187 stimulation. 1, blank; 2, PMA plus A23187; 3, PMA plus A23187 plus purple bamboo salt (1 mg/mL).

in a comparison with the saline-administrated group (Fig. 4). After 2 days, the immobility time was decreased in the purple bamboo salt-administration group (68.6± 15.0 s for 1 g/kg) in comparison with the control group (85.3±17.8 s). After 7 days, the immobility time was

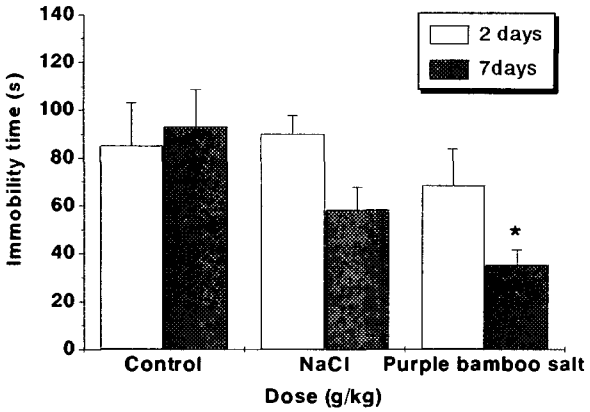

Fig. 4. Effect of purple bamboo salt on FST.
Immobility time recorded during 4 min in the FST in mouse given saline (control group, n=5) or purple bamboo salt (n=5). Data represent mean±SEM of five independent experiments. *$p<0.05$; indicates significant difference from the saline group.

significantly decreased in the purple bamboo salt-administration group (35.4±5.9 s for 1 g/kg) in comparison with the control group (93.2±15.4 s). Our results suggest that the decrease in the immobility time caused by purple bamboo salt administration in the FST might be mediated through immune-enhancement. Because purple bamboo salt decrease the immobility time in this test without stimulating motor activity, we postulate that purple bamboo salt has a potential activity for inducing immune-enhancement.

In order to clarify its mechanisms, we assessed the levels of several blood biochemical parameters in mice after FST. The LDH is known to be accurate indicators of muscle damage and catalyzes the interconversion of pyruvate and lactate (30,31). Therefore, the LDH level increases immediately after exercise. On the other hand the Glc level decreases immediately after exercise. Interestingly, in the present study, the LDH level was

Table 4. Effect of purple bamboo salt on blood biochemical parameters in mice[1)]

	Saline	NaCl (1 g/kg/day)	Purple bamboo salt (1 g/kg/day)
Glc (mg/dL)	252.3±28.4	255.3±28.1	264.7±19.7
LDH (U/L)	575.3±142.6	679.0±108.6	523±61.0
BUN (mg/dL)	17.1±4.2	13.5±0.7	14.8±3.4
CK (mg/dL)	0.6±0.1	0.6±0.1	0.6±0.0
TP (g/dL)	4.4±0.2	4.5±0.1	4.8±0.2

[1)]Purple bamboo salt or NaCl (1 g/kg for 7 days) was administered orally to mice, we have measured the levels of Glc, LDH, BUN, CK and TP in the serum (n=5). Each level was determined by the autoanalyzer. Data represent mean±SEM of five independent experiments.

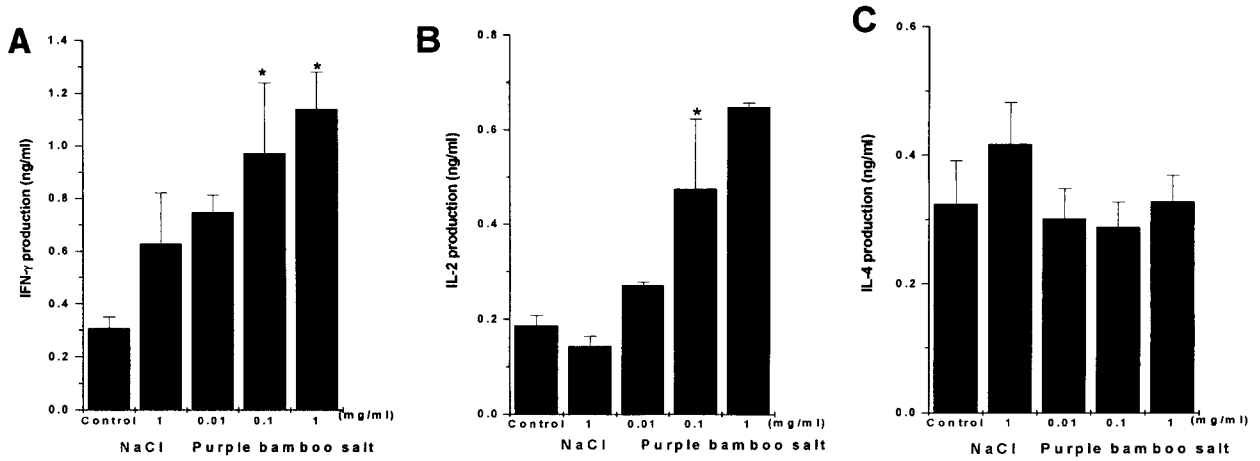

Fig. 5. Effect of purple bamboo salt on IFN-γ, IL-2 and IL-4 production in the MOLT-4 cells.
Culture supernatant was collected from none or purple bamboo salt treated MOLT-4 cells, which were cultured for 24 h. Cytokines levels in culture supernatant was measured using ELISA. Data represent mean±SEM of three independent experiments. *$p<0.05$; significantly different from the saline value.

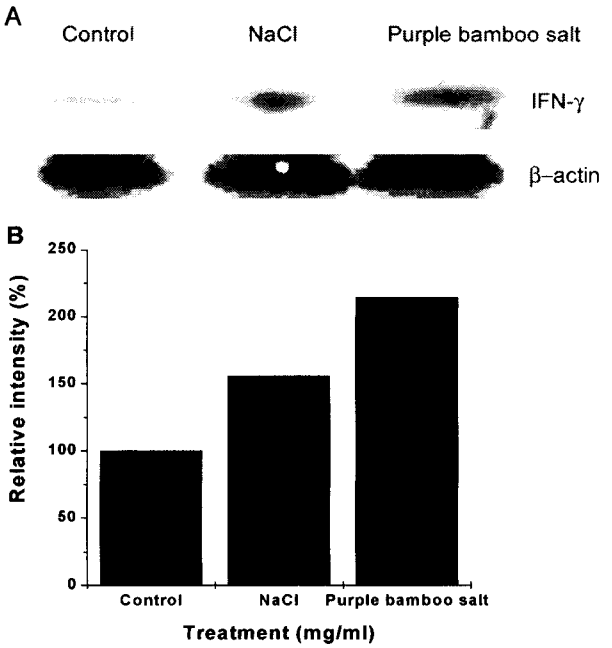

Fig. 6. Effect of purple bamboo salt on IFN-γ expression in the MOLT-4 cells.
The cells were incubated for 24 h in the presence of NaCl (1 mg/mL), purple bamboo salt (1 mg/mL). The protein extracts were prepared and samples were analyzed for IFN-γ expression by western blotting as described method (A). IFN-γ levels were quantitated by densitometry (B).

decreased and Glc level was increased by administration of purple bamboo salt (1 g/kg) (Table 4). These facts suggest that purple bamboo salt may act as energy source. However, the CK and TP level showed no change as compared with those of the saline-administrated mice.

To assess the effects of purple bamboo salt on the production of cytokines, the MOLT-4 cells were treated with various concentrations of purple bamboo salt for 24 h. The levels of IFN-γ, IL-2 and IL-4 were analyzed by ELISA method. As shown in Fig. 5A and 5B, purple bamboo salt (1 mg/mL) significantly increased the interferon (IFN)-γ and IL-2 level compared with media control (about 3.7-fold for IFN-γ, about 3.5-fold for IL-2, $p<0.05$) at 24 h. However, purple bamboo salt did not affect the production of IL-4 compared with media control (Fig. 5C). Purple bamboo salt increased the protein expression of IFN-γ in MOLT-4 cells (Fig. 6). Therefore, we can speculate that IFN-γ and IL-2 increased by purple bamboo salt may contribute to immune-enhancement response. Many cancer vaccines, particularly in combination with immune adjuvants, elicit strong cellular immune responses leading to the production of Th1 type cytokines such as IFN-γ, IL-2, TNF-α (44). Previously we reported that Th2 cytokines levels were higher than Th1 cytokines levels in various diseases including cerebral infarction, allergy and asthma (45,46). Purple bamboo salt may be useful therapy for patients who need enhanced Th1 immune response.

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REFERENCES

1. Kim JB, Paik DI, Moon HS, Jin BH, Park DY. 1993. A

study on the effects of cetylpyridinim chloride and bamboo salt containing mouth rinse on the number of streptococcus mutans and plaque index. *J Kor Acad Dent Health* 17: 176-186.
2. Min BS, Choi HY, Choi YJ, Hong JP, Chun YH, Kang NH. 1995. The reducing effects on dental plaque formation and gingiritis of toothpastes containing bamboo salt and several herb medicines. *J Kor Dent Ass* 33: 65-71.
3. Yang JS, Kim OH, Chung SY, Yoo TM, Roh YN, Yi SY, Chung MW, Ahn MR, Choi HJ, Rheu HM. 1999. Pharmacological evaluation of bamboo salt. *J Appl Pharm* 7: 178-184.
4. Huh K, Kim YH, Jin DQ. 2001. Protective effect of an aged garlic-bamboo salt mixture on the rat with the alcohol-salicylate induced gastropathy. *Yakhak Hoeji* 45: 258-268.
5. Wasserman SI, Marquardt DL. 1998. *Anaphylaxis in allergy: Principles and Practice*. 3rd ed. C. V. Mosby, St. Louis. p 1365.
6. Hua XY, Back SM, Tam EK. 1996. Substance P enhances electrical field stimulation-induced mast cell degranulation in rat trachea. *America Journal of Physiology* 270: L985-991.
7. Shin BK, Lee EH, Kim HM. 1997. Suppression of L-histidine decarboxylase mRNA expression by methyleugenol. *Biochemical and Biophysical Research Communications* 232: 188-191.
8. Sudo N, Tanaka K, Koga Y, Okumura Y, Kubo C, Nomoto K. 1996. Extracellular ATP activates mast cells via a mechanism that is different from the activation induced by the cross-linking of Fc receptors. *Journal of Immunology* 156: 3970-3979.
9. Pearce FL. 1989. Mast cells: function, differentiation and activation. *Current Opinion in Immunology* 1: 630-636.
10. Kim HM, Lee YH, Chae HJ, Kim HR, Baek SH, Lim KS, Hwang CY. 1999. Shini-san inhibits mast cell-dependent immediate-type allergic reactions. *American J of Chinese Medicine* 27: 377-386.
11. Na HJ, Jeong HJ, Bae H, Kim YB, Park ST, Yun YG, Kim HM. 2002. Tongkyutang inhibits mast cell-dependent allergic reactions and inflammatory cytokines secretion. *Clinica Chimica Acta* 319: 35-41.
12. Yi JM, Hong SH, Lee HJ, Won JH, Kim JM, Jeong DM, Baek SH, Lim JP, Kim HM. 2002. Ixeris dentata green sap inhibits both compound 48/80-induced anaphylaxis-like response and IgE-mediated anaphylactic response in murine model. *Biological and Pharmaceutical Bulletin* 25: 5-9.
13. Petersen LJ, Mosbech H, Skov P. 1996. Allergen-induced histamine release in intact human skin *in vivo* assessed by skin microdialysis technique: Characterization of factors influencing histamine releasability. *J Allergy Clin Immunol* 97: 672-679.
14. Segal DM, Taurog J, Metzger H. 1977. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc Natl Acad Sci USA* 74: 2993-2997.
15. Metzger H, Alcaraz G, Hohman R, Kinet JP, Pribluda V, Quarto R. 1986. The receptor with high affinity for immunoglobulin E. *Annu Rev Immunol* 4: 419-470.
16. Alber G, Miller L, Jelsema C, Varin-Bland N, Metzger H. 1991. Structure/function relationships in the mast cell high-affinity receptor for IgE (Fc ε RI): Role of cytoplasmic domains. *J Biol Chem* 266: 22613-22620.
17. Katayama S, Shionoya H, Ohtake S. 1978. A new method for extraction of extravasated dye in the skin and the influence of fastine stress on passive cutaneous allergy in guinea pigs and rats. *Microbiol Immunol* 22: 89-101.
18. Wershil BK, Merkori YA, Murakame T, Galli SJ. 1987. ^{125}I-fibrin deposition in IgE dependent immediate hypersensitivity reaction in mouse skin. Demonstration of the role of mast cells using mast cell-deficient mice locally reconstituted with cultured mast cells. *J Immunol* 139: 2605-2614.
19. Galli SJ. 1993. New concepts about the mast cell. *N Engl J Med* 328: 257-265.
20. Porsolt RD, Bertin A, Jalfre M. 1977. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 229: 327-336.
21. Borsini F, Cesana R, Vidi A, Mennini T. 1991. Evidence that imipramine activates 5-HT1C receptor function. *Eur J Pharmacol* 203: 359-363.
22. Delbende C, Tranchand Bunel D, Tarozzo G, Grino M, Oliver C, Mocaer E, Vaudry H. 1994. Effect of chronic treatment with the antidepressant tianeptine on the hypothalamo-pituitary-adrenal axis. *Eur J Pharmacol* 251: 245-251.
23. Connor TJ, Kelly JP, Leonard BE. 1998. Forced swim test-induced endocrine and immune changes in the rat: effect of subacute desipramine treatment. *Pharmacol Biochem Behav* 59: 171-177.
24. Dubovik BV, Bogomazov SD. 1987. Multifactorial method for assessing the physical work capacity of mice. *Farmakol Toksikol* 50: 116-121.
25. Moriura T, Matsuda H, Kubo M. 1996. Pharmacological study on Agkistrodon blomhoffii blomhoffii BOIE. V. anti-fatigue effect of the 50% ethanol extract in acute weight-loaded forced swimming-treated rats. *Biol Pharm Bull* 19: 62-66.
26. Deyama T, Nishibe S, Nakazawa Y. 2001. Constituents and pharmacological effects of Eucommia and Siberian ginseng. *Acta Pharmacol Sinica* 12: 1057-1070.
27. Kim KM, Yu KW, Kang DH, Suh HJ. 2002. Anti-stress and anti-fatigue effect of fermented rice bran. *Phytother Res* 16: 700-702.
28. Novelli GP, Falsini S, Bracciotti G. 1991. Exogenous glutathione increases endurance to muscle effort in mice. *Pharmacol Res* 2: 149-155.
29. Ozturk N, Husnu Can Baser K, Aydin S, Ozturk Y, Calis I. 2002. Effects of Gentiana lutea ssp. symphyandra on

the central nervous system in mice. *Phytother Res* 7: 627-631.
30. Burr JR, Reinhart GA, Swenson RA, Swaim SE, Vaughn DM, Bradley DM. 1997. Serum biochemical values in sled dogs before and after competing in long-distance races. *J Am Vet Med Assoc* 211: 175-179.
31. Coombes JS, McNaughton LR. 2000. Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. *J Sports Med Phys Fitness* 40: 240-246.
32. Dorchy H. 2002. Sports and type I diabetes: personal experience. *Rev Med Brux* 23: A211-217.
33. Mosmann TR, Coffman RL. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 7: 145-173.
34. Chtanova T, Mackay CR. 2001. T cell effector subsets: extending the Th1/Th2 paradigm. *Adv Immunol* 78: 233-266.
35. Kang BY, Chung SW, Lim YS, Kim EJ, Kim SH, Hwang SY, Kim TS. 1999. Interleukin-12-secreting fibroblasts are more efficient than free recombinant interleukin-12 in inducing the persistent resistance to Mycobacterium avium complex infection. *Immunology* 97: 474-480.
36. Dredge K, Marriott JB, Todryk SM, Dalgleish AG. 2002. Adjuvants and the promotion of Th1-type cytokines in tumour immunotherapy. *Cancer Immunol Immunother* 51: 521-531.
37. Cohn L, Ray A. 2000. T-helper type 2 cell-directed therapy for asthma. *Pharmacol Ther* 88: 187-196.
38. Abbas AK, Murphy KM, Sher A. 1996. Functional diversity of helper T lymphocytes. *Nature* 383: 787-793.
39. Jippo-Kanemoto T, Kasugai T, Yamatodani A, Ushio H, Mochizuki T, Tohya K, Kimura M, Nishimura M, Kitamura Y. 1993. Supernormal histamine release and normal cytotoxic activity of Beige rat mast cells with giant granules. *International Archives Allergy and Immunology* 100: 99-106.
40. Yurt RW, Leid RW, Austen KF. 1977. Native heparin from rat peritoneal mast cells. *J of Biological Chemistry* 252: 518-521.
41. Katayama S, Shionoya H, Ohtake S. 1978. A new method for extraction of extravasated dye in the skin and the influence of fastine stress on passive cutaneous allergy in guinea pigs and rats. *Microbiol Immunol* 22: 89-101.
42. Tasaka K, Mio M, Okamoto M. 1986. Intracellular calcium release induced by histamine releasers and its inhibition by some antiallergic drugs. *Ann Allergy* 56: 464-469.
43. Stenvinkel P. 2001. Inflammatory and atherosclerotic interactions in the depleted uremic patients. *Blood Purif* 19: 53-61.
44. Dalgleish AG. 2000. Cancer vaccines. *Br J Cancer* 82: 1619-1624.
45. Kim HM, Shin HY, Jeong HJ, et al. 2000. Reduced IL-2 but elevated IL-4, IL-6 and IgE serum levels in patients with cerebral infarction during the acute stage. *J Mol Neurosci* 14: 191-196.
46. Jeong HJ, Kim BS, Kim KS, Kim HM. 2002. Regulatory effect of cytokine production in asthma patients by SOOJI CHIM (Koryo Hand Acupuncture Therapy). *Immunopharmacol Immunotoxicol* 24: 265-274.