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Antiobesity Effects of Bamboo Salt in C57BL/6 Mice

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ABSTRACT Antiobesity effects of bamboo salt (BS) were evaluated compared with those of purified salt and solar salt by oral administration in a diet-induced obesity model using C57BL/6 mice. Compared with other salts, BS, especially nine times baked BS (BS-9×), significantly reduced body weight, food efficiency ratio, and weights of epididymal adipose tissue and liver in high-fat diet–fed mice. Furthermore, BS suppressed the expression of adipogenic factors, such as CCAAT/enhancer binding protein alpha (C/EBPα), peroxisome proliferator-activated receptor gamma (PPARγ), and sterol regulatory element-binding protein 1c (SREBP-1c). Therefore, BS may suppress obesity by downregulating adipogenesis.

KEY WORDS: • *antiobesity* • *bamboo salt* • *C57BL/6 mice*

Energy intake beyond an individual's energy expenditure is the main cause of obesity, which is known to lead to the accumulation of energy into adipose tissues for maintenance of metabolic balance.[1,2] Obesity is a significant public health problem and also leads to chronic diseases, such as diabetes, hypertension, brain stroke, cardiac myoinfarction, and cancer.[3–5]

Bamboo salt (BS), especially purple BS (BS-9×), is a specially processed salt based on a traditional recipe in Korea using solar salt (SS) and a bamboo cylinder. It is different from common salt (purified salt [PS] and SS) and has abundant concentrations of minerals, such as potassium, calcium, and sulfur.[6] It has been found to have therapeutic effects on diseases, such as dental plaque, gastropathy, certain types of allergic reactions, and cancer.[6–12] In this study, to the best of our knowledge, we examined the antiobesity effects of BS for the first time. We used C57BL/6 mice to observe the effects of BS on changes in body weight, weights of adipose tissue and liver, and adipogenic gene expression.

BS once baked (BS-1×), three times baked (BS-3×), and nine times baked (BS-9×), and SS were provided by Taesung Food Company (Gochang, Jeonbuk, South Korea). PS manufactured by Hanju Corporation (Ulsan, South Korea) was purchased at a local market (Jang Jeon-dong, Geum Jeong-gu, Busan, South Korea). Based on the results of a safety test by Kim *et al.*,[13] salts were dissolved in sterilized distilled water, and each salt solution was administered to mice at dosages of 2727 (high) and 1363 (low) mg/kg/day, which corresponded to daily administrations of 10 and 5 g of salt for a 60 kg man, respectively.[14]

Under approval by the Pusan National University Institutional Animal Care and Use Committee (PNU-2012-0128), 6-week-old C57BL/6 mice ($n = 119$) were used in this animal experiment. The animals were divided into groups of seven mice each after a 1-week stabilization period. The experimental design was as follows: chow diet (normal), high-fat diet (HFD), HFD + PS (PS; high, low), HFD + SS (SS; high, low), HFD + BS-1× (BS-1×; high, low), HFD + BS-3× (BS-3×; high, low), and HFD + BS-9× (BS-9×; high, low). Each prescribed saline solution was orally administered to each mouse of the designated experimental group by gavage for 8 weeks, whereas rodent feed, HFD or chow diet, and water were provided *ad libitum*. In all experiments, body weights and amounts of feed consumed were measured twice a week using a top-loading balance.

At the end of the 8-week period, all the mice were sacrificed without fasting.[15] Their tissues were harvested, weighed, snap-frozen in liquid nitrogen, and stored at −80°C until used. Blood collected from abdominal aortas underwent plasma separation by centrifugation and stored at −80°C until analysis.

Liver and adipose tissues were immersed into Trizol and subjected to homogenization on ice. mRNA extraction and cDNA constitution of these tissues were performed by a modified method of Do *et al.*[16] Amplification was performed in a denaturing phase at 94°C for 1 min, an annealing phase at 54°C for 1 min, and an extension phase at 72°C for 30 sec, which constituted one cycle. After 40 amplification cycles, a finalization phase at 72°C for 7 min was carried out. The amplified PCR products were run on 1.0% agarose gels, stained with ethidium bromide, and visualized under UV light.

As shown in Figure 1, changes in body weight of mice, especially those of the high-dosage group, were reduced.

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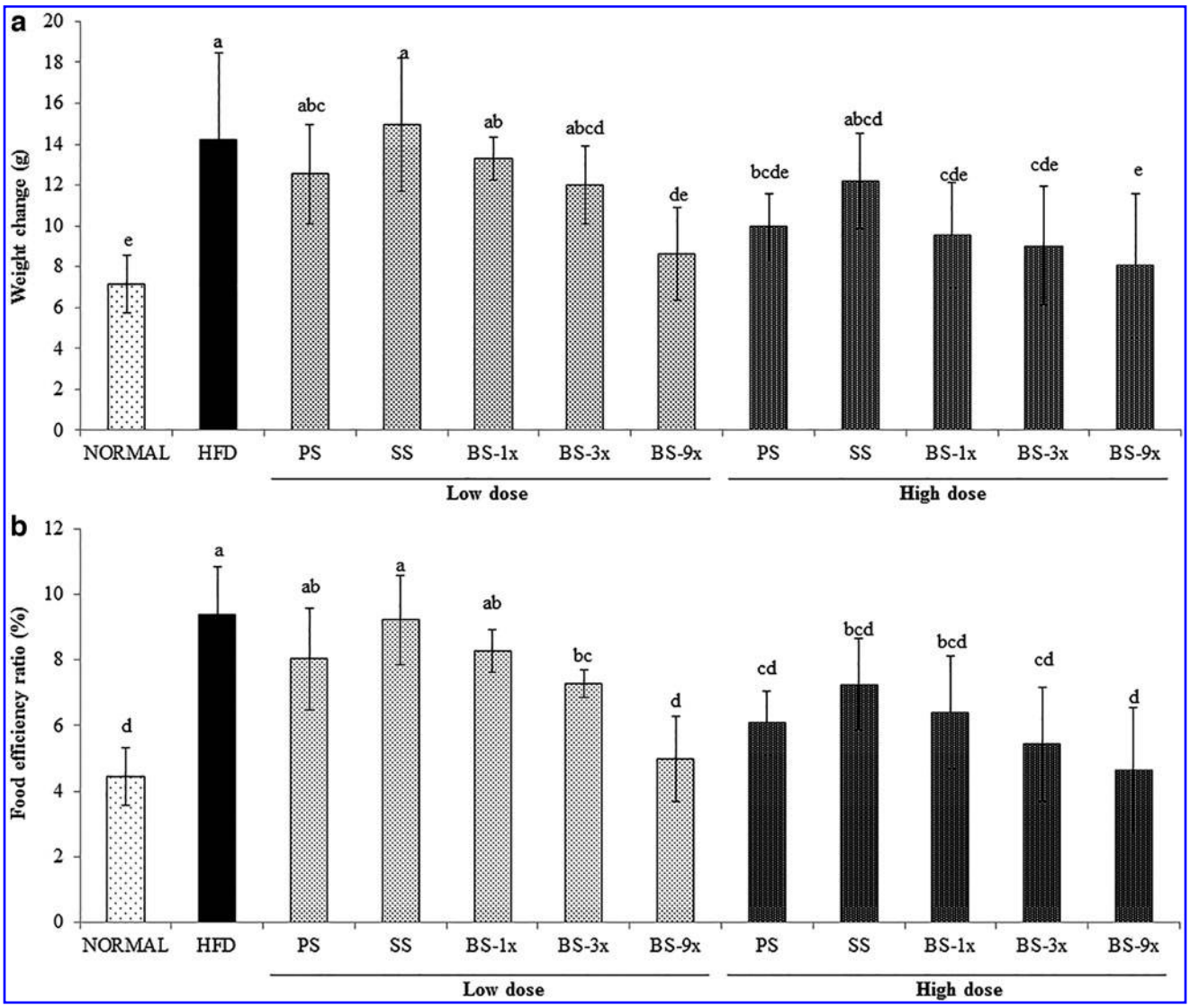

FIG. 1. Changes in **(a)** body weight change (final weight − initial weight) and **(b)** food efficiency ratio (body weight/total feed consumption) upon administration of salt. [abcde]Mean values with different superscript letters over the bars are significantly different ($P < .05$) according to Duncan's multiple range test.

Addition of a salt sample significantly reduced the span of weight change compared with the HFD group ($P < .05$). Especially, the weight change in the BS-9× group was the smallest in the salt-administered groups, and it was similar to that of the normal diet group (Fig. 1a). Food efficiency ratio, which represents the changes in body weight divided by the consumed feed in a certain period, of mice was also suppressed. Among the salt-administered groups, the BS-9× group showed the lowest food efficiency ratio (4.65%), which was significant compared with that of the HFD group ($P < .05$; Fig. 1b). Among all experimental groups, average weight of epididymal adipose tissue (EAT) in the BS-administered group was significantly lower compared with that of the HFD group ($P < .05$). Especially, the BS-9× group showed the lowest weight of EAT among all salt-administered groups (1.00 g; Fig. 2).

Liver weight was also measured, and average weight of liver in the BS-administered group was significantly lower compared with that of the HFD group. Especially, the average liver weight of the BS-9× group was lowest among all salt-administered groups (1.21 g), although the significance was low compared with other feature changes (Fig. 3). We also measured serum leptin concentrations of the experimental groups (2727 mg/kg/day). Administration of BS, including BS-9×, was found to significantly lower serum leptin concentrations compared with the HFD group (28.05 ng/mL; Fig. 4).

Figure 5 shows changes in hepatic mRNA expression. Expression levels of sterol regulatory element-binding protein 1c (SREBP-1c) and CCAAT/enhancer binding protein alpha (C/EBPα) were prominently lower in groups administered BS. Expression of peroxisome proliferator-activated

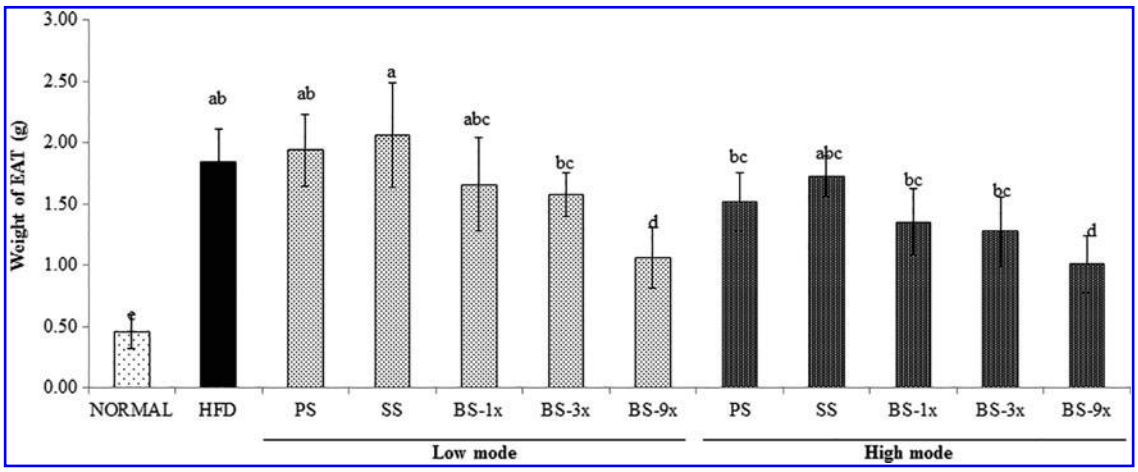

FIG. 2. Changes in weight of epididymal adipose tissue (EAT) upon administration of salt. [abcd]Mean values with different superscript letters over the bars are significantly different ($P < .05$) according to Duncan's multiple range test.

receptor gamma (PPARγ) was also lower with administration of BS as well. Especially, the lowering of expression of *C/EBP*α and *SREBP-1c* was most obvious in the BS-9× group (Fig. 5a). We also observed changes in mRNA expression from EAT. BS-administered groups showed suppressed expression of *PPAR*γ and *C/EBP*α compared with the HFD group. Especially, the expression of PPARγ was profoundly diminished in the BS-9× group (0.20 time; Fig. 5b).

This study evaluated the antiobesity effects of BS, which were mediated by reducing changes in total body weight and food efficiency ratio. Body weight changes in the BS groups were lower than that in the SS group, and they decreased as baking time of BS increased. Furthermore, changes in food efficiency ratio in the BS groups occurred in the same manner as the changes in body weight. It may be inferred that BS suppressed obesity without changes in dietary intake based on reduction of adipose tissues weights, including EAT. Actually, the ratio of EAT to the total weight was significantly reduced in the BS-9× group compared with the HFD group (data not shown).

BS has been found to contain more hydrogen sulfide (H_2S)-releasing substances than any other salts.[17] H_2S, endogenous or meal-derived, is known to normalize lipid metabolism and to prevent hepatosteatosis, even if the related mechanism is not fully understood.[18] Accumulation of fat into adipose tissues was found to promote oxidative stress by many processes, including reactive oxygen species production, whereas its attenuation was found to lead to improvement of metabolic syndrome.[19] In addition, intake of minerals, such as calcium, potassium, and phosphorus, has been found to suppress obesity.[20–22] Furthermore, BS was found to have higher reduction potential, more -OH groups, and more mineral compounds (potassium, calcium, phosphorus, and magnesium) than PS and SS,[23] which may also account for its antiobesity effects we observed.

Leptin is an adipokine present in amounts proportional to the amount of accumulated triacylglycerol (TG).[24] This study showed that application of BS *in vivo* significantly reduced leptin concentrations, suggesting BS may have suppressed accumulation of TG inside the adipose tissue.

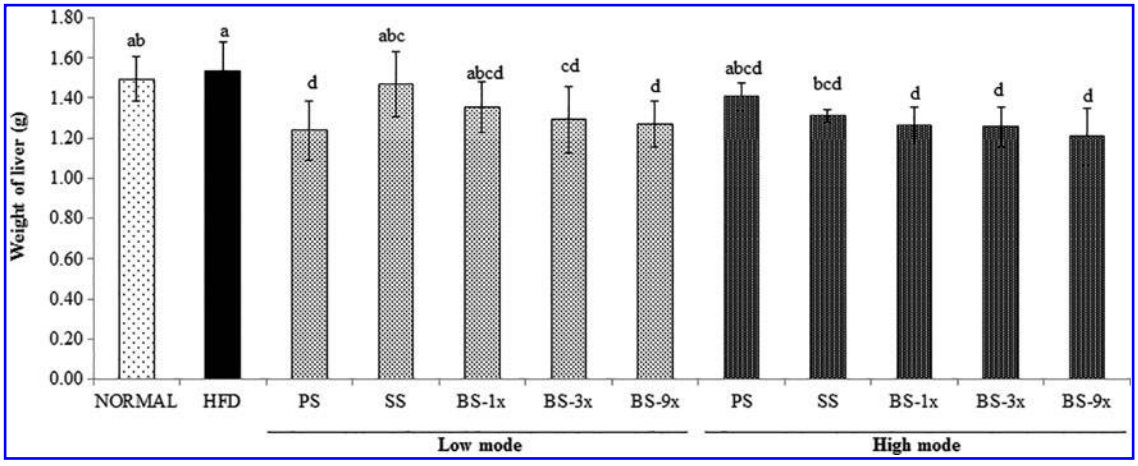

FIG. 3. Changes in liver weight upon administration of salt. [abcd]Mean values with different superscript letters over the bars are significantly different ($P < .05$) according to Duncan's multiple range test.

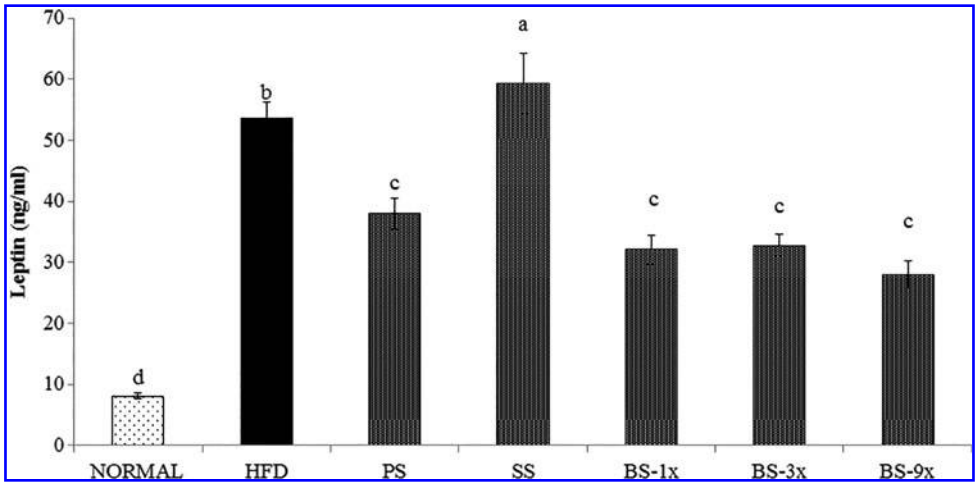

FIG. 4. Changes in serum leptin concentration upon administration of salt. [abcd]Mean values with different superscript letters over the bars are significantly different ($P < .05$) according to Duncan's multiple range test.

Kim *et al.*[13] reported that administration of BS to rats has no significant toxicological effects on hepatic factors, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In our observations, liver weight of every BS-treated group (especially the BS-9× group) was lighter than that of other groups, although such changes were less significant than those of body weight. Suppression of hepatic lipid accumulation by BS can be explained by our histological analysis, and ALT and AST levels in the BS-treated group were also lower than those in the normal group (data not shown).

PPARγ and C/EBPα, alone or in cooperation by positive feedback, induce the transcription of many adipogenic genes that eventually encode proteins and enzymes involved

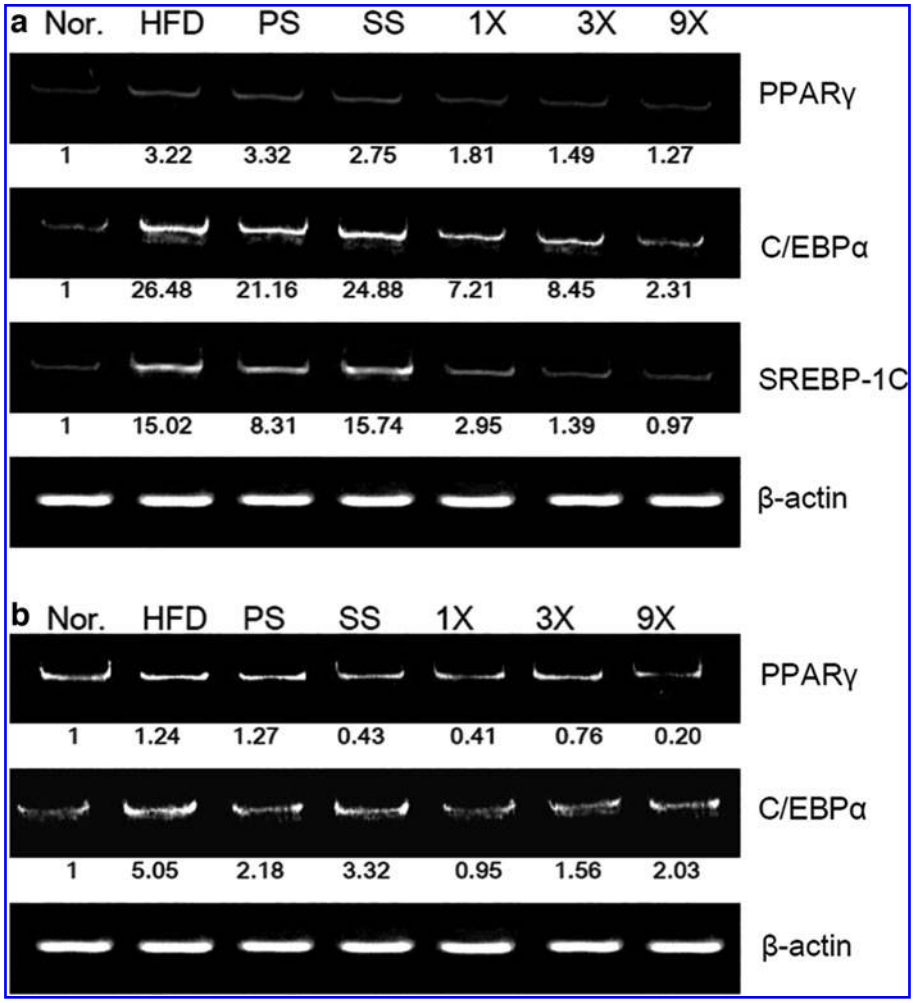

FIG. 5. Changes in mRNA expression on **(a)** liver and **(b)** EAT upon administration of salt. The *numbers* below the relevant bands stand for relative expression ratio to normal diet within a certain factor.

in adipogenesis of certain tissues.[25,26] Especially, PPARγ promotes expression of the transcription factor SREBP-1c, which controls lipogenic factors related to fatty acid synthesis.[27,28] Based on the above facts, administration of BS might have contributed to suppression of these adipogenic genes, eventually reducing accumulation of fat in the liver and EAT. As all substances absorbed in organs such as the small intestine initially pass through the liver by the hepatic portal vein for processing before entrance into the systemic circulation,[29] BS might have initially affected the liver such that lipogenesis and adipogenesis were suppressed. It was also reported that BS promotes expression of β-oxidation-related hepatic factors, such as *PPAR*α and *CPT-1*, in a C57BL/6 diet-induced obesity (DIO) model,[30] which may support the antiobesity effects of BS.

In conclusion, the administration of BS prevented obesity by suppressing adipogenesis in an animal model. Detailed research on the antiobesity effects of BS administration in a murine DIO is ongoing.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM: Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *J Am Med Assoc* 2004;291:2847–2850.
2. Akoh CC: Lipid-based fat substitutes. *Crit Rev Food Sci Nutr* 1995;35:405–430.
3. Xavier F, Sunyer PI: The obesity epidemic: Pathophysiology and consequences of obesity. *Obes Res* 2002;10:97–104.
4. Lee WJ, Koh EH, Won JC, *et al.*: Obesity: The role of hypothalamic AMP-activated protein kinase in body weight regulation. *Int J Biochem Cell Biol* 2005;37:2254–2259.
5. Giri S, Rattan R, Haq E, *et al.*: AICAR inhibits adipocyte differentiation in 3T3-L1 and restores metabolic alterations in diet-induced obesity mice model. *Nutr Metab* 2006;3:31–50.
6. Zhao X, Song JL, Jung OS, Lim YI, Park KY: Chemical properties and *in vivo* gastric protective effects of bamboo salt. *Food Sci Biotechnol* 2014;23:895–902.
7. Kim JB, Paik DI, Moon HS, Jin BH, Park DY: A study on the effects of cetylpyridinim chloride and bamboo salt containing mouth rinse on the number of *streptococcus mutans* and plaque index. *J Korean Acad Dent Health* 1993;17:176–186.
8. Min BS, Choi HY, Choi YJ, Hong JP, Chun YH, Kang NH: The reducing effects on dental plaque formation and gingivitis of toothpastes containing bamboo salt and several herb medicines. *J Korean Dent Assoc* 1995;3:65–71.
9. Yang JS, Kim OH, Chung SY, *et al.*: Pharmacological evaluation of bamboo salt. *J Appl Pharm* 1999;7:178–184.
10. Huh K, Kim YH, Jin DQ: Protective effect of an aged garlic-bamboo salt mixture on the rat with the alcohol-salicylate induced gastropathy. *Yakhak Hoeji* 2001;45:258–268.
11. Shin HY, Na HJ, Moon PD, *et al.*: Inhibition of mast cell-dependent immediate-type hypersensitivity reactions by purple bamboo salt. *J Ethnopharmacol* 2004;91:153–157.
12. Zhao X, Ju J, Kim HM, Park KY: Antimutagenic activity and *in vitro* anticancer effects of bamboo salt on HepG2 human hepatoma cells. *J Environ Pathol Toxicol Oncol* 2013;32:9–20.
13. Kim JG, Seo KW, Lee BH, *et al.*: 3 months repeated dose toxicity studies of the bamboo salt (Jukyum) in rats. *J Toxicol Public Health* 2002;18:149–157.
14. Center for Drug Evaluation and Research (U.S.). *Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers.* Rockville, MD: U.S. Dept. of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 2005. http://purl.access.gpo.gov/GPO/LPS119812 (accessed on April 1, 2013).
15. Horton JD, Bashmakov Y, Shimomura I, Shimano H: Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci USA* 1998;95:5987–5992.
16. Choi BH, Ahn IS, Kim YH, *et al.*: Berberine reduces the expression of adipogenic enzymes and inflammatory molecules of 3T3-L1 adipocyte. *Exp Mol Med* 2006;38:599–605.
17. Zhu J: *Effects of various salts on aging in D-Galactose-induced aging rats* [MS thesis]. Mokpo National University, Mokpo, Jeonlanam-do, Korea, 2013.
18. Mani S, Cao W, Wu L, Wang R: Hydrogen sulfide and the liver. *Nitric Oxide* 2014;15:62–71.
19. Furukawa S, Fujita T, Shimabukuro M, *et al.*: Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004;114:1752–1761.
20. Schrager S: Dietary calcium intake and obesity. *J Am Board Fam Pract* 2005;18:205–210.
21. Mariosa LS, Ribeiro-Filho FF, Batista MC, *et al.*: Abdominal obesity is associated with potassium depletion and changes in glucose homeostasis during diuretic therapy. *J Clin Hypertens* 2008;10:443–449.
22. Obeid OA: Low phosphorus status might contribute to the onset of obesity. *Obes Rev* 2013;14:659–664.
23. Zhao X, Jung OS, Park KY: Alkaline and antioxidant effects of bamboo salt. *J Korean Soc Food Sci Nutr* 2012;41:1301–1304.
24. Havel PJ: Role of adipose tissue in body-weight regulation: Mechanism regulation leptin production and energy balance. *Proc Nutr Soc* 2000;59:359–371.
25. Shao D, Lazar, MA: Peroxisome proliferator activated receptor gamma, CCAAT/enhancer-binding protein alpha, and cell cycle status regulate the commitment to adipocyte differentiation. *J Biol Chem* 1997;272:21473–21478.
26. Gregoire FM, Smas CM, Sul HS: Understanding adipocyte differentiation. *Physiol Rev* 1998;78:783–809.
27. Rosen ED, Hsu CH, Wang X: C/EBPα induces adipogenesis through PPARγ: A unified pathway. *Genes Dev* 2002;16:22–26.
28. Nogalska A, Sucajtys-Szulc E, Swierczynski J: Leptin decreases lipogenic enzyme gene expression through modification of SREBP-1c gene expression in white adipose tissue of aging rats. *Metabolism* 2005;54:1041–1047.
29. Eipel C, Abshagen K, Vollmar B: Regulation of hepatic blood flow: The hepatic arterial buffer response revisited. *World J Gastroenterol* 2010;16:6046–6057.
30. Ju JH, Song JL, Moon SH, Park KY: Anti-obesity effect of bamboo salt on diet-induced obesity in C57BL/6 mice. Experimental biology.org http://experimentalbiology.org/2014/Program-Information/Society-Programs/Posters-Monday.aspx (accessed in May 2014).