

## Surprisingly, traditional purple bamboo salt, unlike other salts does not induce hypertension in rats

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### Abstract

Hypertension induces many of the social costs related by cardiovascular diseases. Sodium is known as a crucial factor in inducing type I hypertension. In traditional Korean medicine, bamboo salt (BS) has been used in the attenuation of salts toxic coldness and nowadays it has shown various therapeutic effects. It contains mostly sodium chloride (about 91.7% of BS); however, the effect of BS on hypertension is still not completely understood. Thus, we investigated the effect of BS on blood pressure for the first time. Two group of BS, sun-dried salt (SDS), NaCl, or distilled water (DW, vehicle control) was administrated orally for 8 weeks. Although BS had no effect on body weight and food intake, it increased water intake ( $p < 0.05$ ). The BS groups, in terms of blood pressure, was similar to the DW group; whereas the SDS and NaCl groups showed significantly increased blood pressure levels ( $p < 0.05$ ). BS also decreased sodium-chloride cotransporter (NCC) mRNA expression, unlike SDS or NaCl. These observations indicate that BS may be a promising strategy for the prevention of various diseases including salt-related diseases.

**Key words** hypertension, blood pressure, sodium chloride, bamboo salts, sodium-chloride cotransporter

### INTRODUCTION

Hypertension means high arterial blood pressure in general. About 90% of over 55-year-old people are near or have higher stage 1 hypertension ( $> 140/90$  mmHg), it seemed to be natural rising blood pressure with aging. And stage 2 ( $> 160/100$  mmHg) hypertension is lower than stage 1 ( $> 140/90$  mmHg) hypertension people in over 70-year-old people (Vasan et al., 2002). Hypertension is divided into primary or essential hypertension and secondary hypertension; primary hypertension is used to describe 90 - 95% of the hypertension cases and occurs due to unclear medical causes, and secondary hypertension is the rest (5 - 10%) of the cases and occurs due to clear medical causes (Carretero and Oparil, 2000). In terms of public health and social costs, hypertension contributes to the occurrence of stroke, myocardial infarction and kidney failure, and finally to death (Mu et al., 2011). A high salt or sodium intake is a major cause of hypertension. Salt increases peripheral vascular resistance and arterial constriction (Blaustein et al., 2012).

The sodium ion is one of the essential ions for human survival, and it is ubiquitous in human fluid. Sodium is usually taken through water and food intake. Most water supplies contain less than 20 mg/l of sodium but its range is 0.4 - 1900 mg/l, and food including vegetables to manufactured food contains sodium from 10 mg/kg up to 20 g/kg in a survey in the USA in 1963 - 1966. In a low-sodium diet, sodium intake needs to be less than 2 g/d and it is recommended 20 mg/l water 2 l/d by the World Health Organization (WHO). LD50 in an acute

exposure of sodium is 1572 mg/kg, 1180 mg/kg, and 3147 mg/kg in mice, rats, and rabbits, respectively. Also, it has been demonstrated clearly that long-term exposure of high sodium intake induces hypertension in different species of animals including experimental animals such as rats. In contrast, long-term exposure of a low-sodium diet, such as 157 mg/kg of salt in the diet, did not show blood pressure change. The major sodium supplement is salts, and they are usually used as seasoning in cooking or in food manufacturing (WHO, 1996).

In Korea, bamboo salts (BS) are mainly ingested for health because they are produced using processes that lead to decreased toxicity and the conversion of acidity to strong alkalinity when compared to sun-dried salts (SDS). According to traditional medical theory, temper of salts is cold and attenuates its cold temper by bamboo's hot temper through baking. In this reason, BS production by traditional method has mean as traditional material. The production of BS is usually as follows: SDS are stuffed inside bamboo and covered with yellow mud, the products are then baked with pine wood and pine resin at about 1000 to 1500°C for 8 - 10 h in a kiln, and then powdered. It is a maximum eight times repeatedly stuffed inside the bamboo and baked. Finally, it is baked at about 1300 to 2000°C. Especially, the nine-time baked bamboo salt is called purple bamboo salts (PBS) for emphasizing, and less than eight-time baked salt called normal BS. PBS changes composition compared with SDS. The contents of iron, silicon, potassium, and phosphate were higher, whereas the sulfate content was lower in the PBS (Shin et al., 2004). BS is reported to have various therapeutic effects on diseases (Huh et al., 2001; Kim et al., 1993; Min et al., 1995; Yang et al., 1999). Here, we demonstrated the effect of BS on blood pressure.

### MATERIALS AND METHODS

#### Animals

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Experiments were performed on Sprague-Dawley rats ( $300 \pm 10$  g). They were housed at a controlled temperature ( $20 \pm 2^\circ\text{C}$ ) and were maintained on 12 h light and 12 h darkness (lights on from 07:00 h to 19:00 h), with food and water made available ad libitum. The rats were divided into 5 groups as follow: distilled water (DW, vehicle control), NaCl, SDS, BS (baked 3 times), and PBS (baked 9 times). Each group consisted of 5 rats and was administrated orally 0.5 g/kg for 8 weeks. Food and water intake was checked every day, and body weight was measured twice a week. Rat care and experimental procedures were performed under approval from the Animal Care Committee of Kyung Hee University.

### BS Preparation

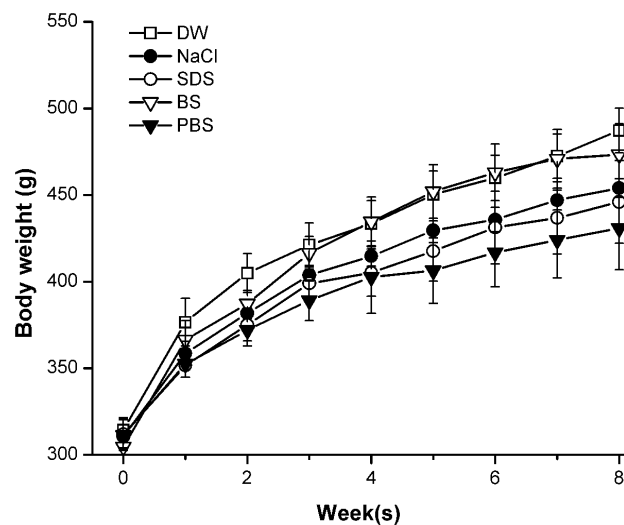
BS was provided from Tae-sung Food Inc. (Jeonbuk, Republic of Korea). It contains about 91.7% sodium chloride. Powdered BS and PBS were dissolved in DW then filtered through a 0.22- $\mu\text{m}$  syringe filter.

### Blood pressure measurement

After 56 days of salt administration, we removed water for 12 h and anesthetized the rats with zoletil 50<sup>®</sup> and rumpun<sup>®</sup> in a 1:2 ratio solution 1 ml/kg. During anesthesia, catheterized in one side of external iliac artery by laboratory PolyE polyethylene non-sterile tubing (#598321, Havard Apparatus, USA) connected iWorx 214 (iworx system, USA) with a research grade pressure transducer (Havard Apparatus, USA), and using LabScribe2 software (iworx system, USA). 5 to 30 min after being stabilized, 1 ml DW was administrated orally by zonde and the blood pressure was observed. Data were gained at 20 Hz.

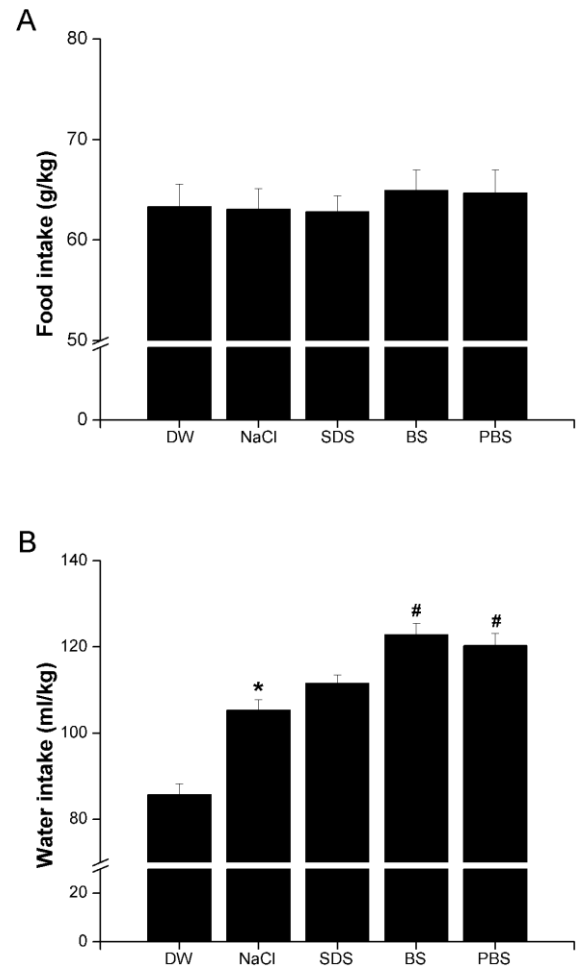
### Reverse transcription-polymerase chain reaction

After the blood pressure was measured, kidney cortex tissue was taken. In brief, total RNA was isolated from the kidney cortex according to the manufacturer's specification using an easy-BLUE<sup>™</sup> RNA extraction kit (iNtRON Biotech, Korea). The concentrations of total RNA in the final elutes were determined by spectrophotometry. Total RNA (2.5  $\mu\text{g}$ ) was heated at  $65^\circ\text{C}$  for 10 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 90 min at  $37^\circ\text{C}$  using a cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). PCR was performed with the following primers: for



**Fig. 1.** Effect of BS and PBS on body weight. Each group was administrated orally 0.5 g/kg for 8 weeks. The body weight was measured twice a week. There was no significance. DW, distilled water; SDS, sun-dried salt; BS, bamboo salt (baked 3 times); PBS, purple bamboo salt (baked 9 times).

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**Fig. 2.** Effects of BS and PBS on food and water intake. (A) Food intake. (B) Water intake ml/kg. It is the mean value of every last 21d intake. DW, distilled water; SDS, sun-dried salt; BS, bamboo salt (baked 3 times); PBS, purple bamboo salt (baked 9 times). \* $p < 0.05$ , significantly different from DW group; # $p < 0.05$ , significantly different from NaCl group.

the rat sodium chloride channel (NCC, F: 5'-TGG CTC ATC ATC CTG CTG TC-3'; R: 5'-GGC TTT GTC CTT AGA TGC TG-3'), rat serine/threonine-protein kinase 4 (WNK4, F: 5'-CTA TCC AGG ATC TTC TGA CCC-3'; and WNK4/R: 5'-TCT TGC TGT TGT GAG TTT GC-3'), and rat  $\beta$ -actin (F: 5'-TTC TAC AAT GAG CTG CGT GTG-3'; and R: 5'-TTC ATG GAT GCC ACA GGA TTC-3') was used to verify whether equal amounts of the RNA were used for reverse transcription and PCR amplification from different experimental conditions. Products were electrophoresed on a 1% agarose gel and visualized by staining with ethidium bromide (Oh et al., 2012).

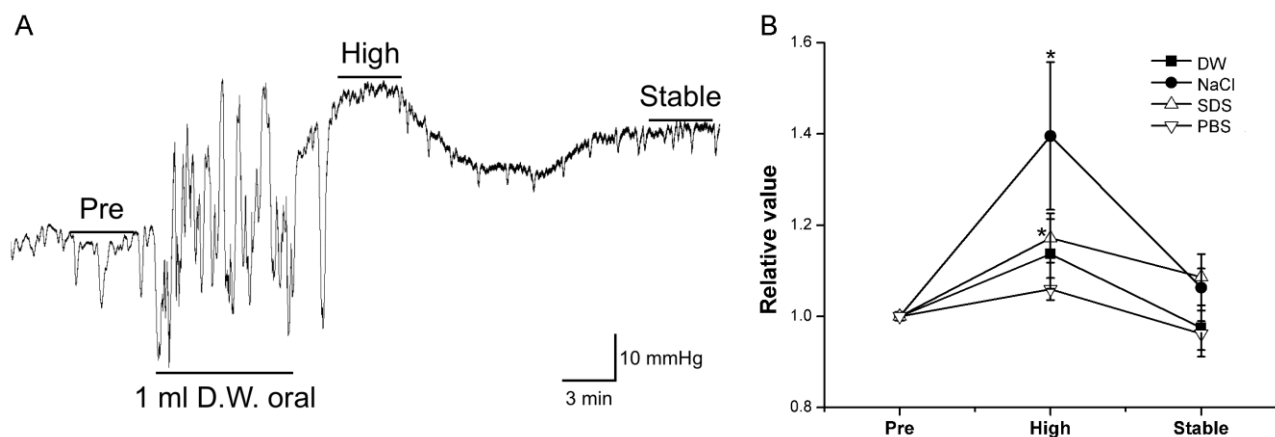
### Statistics

Data were expressed as the mean  $\pm$  standard error of mean (S.E.M). For comparisons between groups, a one-way ANOVA and Duncan's post-hoc test were performed with  $p < 0.05$  as an indication of statistical significance.

## RESULTS

### BS and PBS increased water intake but not food intake

Body weight increased in the experiment but there was no significant difference between each group (Fig. 1). To



**Fig. 3.** Effect of PBS on blood pressure. After anesthesia, found external iliac artery and catheterized heparin filled tube. Recorded blood pressure 10 to 30 min stabilized and 1 ml DW oral administrated. (A) Scheme of blood pressure. (B) Relative blood pressure graph compared to the Pre stage. Pre, Pre-stabilized stage before DW administration; High, DW 1 ml administration after peaked and prolonged stage; Stable, re-stabilized stage 20 min after from High stage. All stages averaged 3 min data. DW, distilled water; SDS, sun-dried salt; PBS, purple bamboo salt (baked 9 times). \* $p < 0.05$ , significantly different from Pre stage.

compensate for each animal's individual differences, individual water and food intake was divided by body weight (ml/kg or g/kg). The rate of food intake was not statistical significant in the NaCl, SDS, BS, and PBS groups (Fig. 2A). However, the rate of water intake in the NaCl-administered group significantly increased compared to the vehicle control group ( $p < 0.05$ ). The BS and PBS groups had significantly more water intake than the NaCl group (Fig. 2B,  $p < 0.05$ ).

#### PBS decreased blood pressure but not NaCl.

During the anesthesia period, DW administration drastically increased blood pressure and then the levels dropped to normal about 20 min after the peak blood pressure. Thus, the time can be divided into three main stages: the pre-stabilized blood pressure stage (Pre) was before DW administration, the high blood pressure stage (High) was blood pressure raising to the peak stage after DW administration, and the re-stabilization stage (Stable) about 20 min after the High stage (Fig. 3A). For reducing individual error, all mean blood pressure levels were converted from the relative value to the Pre stage. In the High stage, the NaCl group's levels most drastically increased, and SDS and PBS were increased slightly. There was no significance in each High stage, but the increases in the NaCl and SDS groups were significant compared to Pre stage. Especially, the PBS group was almost similar to the DW group. But during the Stable stage, all of them did not show significant changes compared to each other and the Pre stage (Fig. 3B). It was shown that with water intake in a high sodium condition, a high NaCl intake had the effect of the largest increase in blood pressure and SDS showed lower levels than NaCl but had a similar effect of NaCl. PBS was shown, like DW, to produce a small increase or a negligible effect on blood pressure. BS group was undergone together but serious individual errors were occurred and failed to get enough data for significant in statistics after DW administration. For this reason, the BS results were discarded for not having enough cases and results for meaningful data.

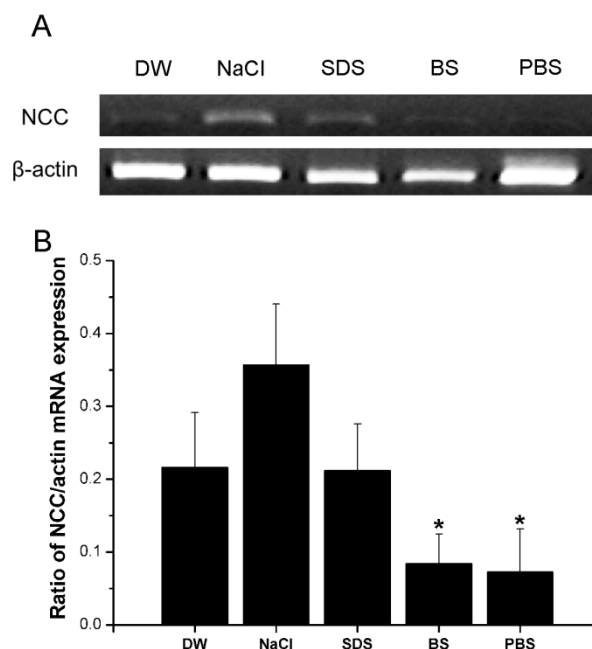
#### BS and PBS inhibited NCC mRNA expression

NCC plays a crucial role in sodium retention in renal distal convoluted tubes. Increase in NCC induces increase of sodium retention, and it is critical in the blood pressure increase. NCC expression in distal convoluted tubes is suppressed by WNK4 (Mu et al., 2011). To investigate the effects of BS and PBS on distal convoluted tubes' NCC mRNA expression, we performed the RT-PCR. The expression of NCC mRNA in the NaCl group

increased compared to the vehicle control group. However, BS and PBS significantly decreased the NCC mRNA expression (Fig. 4,  $p < 0.05$ ). BS, PBS, SDS, and NaCl had no effect on WNK4 mRNA expression (data not shown).

## DISCUSSION

Many cultures have their own useful knowledge through centuries of experience, and some this knowledge develops to folk or traditional medicine. PBS is one of them in the Korean tradition. As described previously, the components of PBS,



**Fig. 4.** Effects of BS and PBS on NCC mRNA expression. (A) NCC mRNA expression levels in the kidney cortex tissue were analyzed by RT-PCR. (B) The levels of NCC mRNA expression were quantified by densitometry. This expression was normalized to the actin expression. DW, distilled water; SDS, sun-dried salt; BS, bamboo salt (baked 3 times); PBS, purple bamboo salt (baked 9 times). \* $p < 0.05$  significantly different from NaCl group.

compared with those of SDS, were changed (Shin et al., 2004). Though it is just small difference in the total portion of salts, PBS is reported to have therapeutic effects for diseases such as viral diseases, dental plaque, gastropathy, diabetes, circulatory organ disorders, cancer, anti-inflammatory disorders, allergic rhinitis, and cisplatin-induced ototoxicity (Min et al., 1995; Shin et al., 2004; Yang et al., 1999; Huh et al., 2001; Sharma et al., 2001; Myung et al., 2011; Jeong et al., 2011; Kim et al., 2012). Nowadays, inflammation is considered important in the cause of hypertension. Its mechanism is that if inflammation is induced in blood vessels, especially in the arteries, then vessel wall fibrosis and wall thickness come together and finally induce vessel stiffness. It means the loss of vessel wall elasticity and the increase of total peripheral resistance, thus inducing hypertension. Also, it induces hypertrophy (Waki et al., 2013; Schreier et al., 2013). It might be suggested that reported anti-inflammatory potential of PBS finally affected reducing blood pressure. In this study, most blood pressure raised group was NaCl administration group, and PBS was almost the same as DW. In this study, we were shown that PBS did not increase after DW administration in Fig. 3. But BS group was not in Fig. 3. It was occurred emergency after DW administration and did not get enough data for significant in statistics. But compared Fig. 4, we thought that effect of BS on hypertension was similar to PBS.

Most PBS-related studies including the present study do not explain why BS has various effects. But one obvious point is that PBS is not a pure material like NaCl. The major component of PBS is NaCl, and SDS is too, but NaCl showed a difference when compared with SDS or PBS. Shin et al. (2004) reported that PBS increases sodium, chloride, potassium, calcium, iron, and manganese ion portions and decreases magnesium, silicon and sulfur compared to SDS. The sodium and chloride are the major components and these other components are just 3% of total salts. With the excluded trace ions and NaCl, the PBS effects are considered to magnesium, potassium, and calcium. These three ions are important factors in inflammation and blood pressure. (Rosanoff et al., 2012; Nielson et al., 2010; Yamori et al., 2011; Yogi et al., 2010). Nevertheless, it is unclear that which ion is the main ion that exerts PBS effectiveness on blood pressure. Further studies will be needed to clarify precisely the mechanism of BS that has the effect on blood pressure.

WNK4 is an important factor and it regulates the activity of a variety of ion channels in kidney involving many of co-transporters and exchangers even those that have no structural relation. In other words, WNK4 is the most important factor related to electrolyte fluxes (Kahle et al., 2004). In hypertension, sodium is a major inducing factor and the most common electrolyte. Also, in electrolyte regulation, the autonomic nervous system plays a crucial role. Adrenergic receptor activation inhibits WNK4 expression and induces NCC expression, and it increases sodium retention in renal distal convoluted tubes. This increase of sodium retention leads to higher blood pressure in the end (Mu et al., 2011). We thus postulate that the inhibition of NCC expression by BS and PBS would be an autonomic nervous system independent mechanism. One possible suggestion is that BS and PBS would "wash out" the sodium. The BS and PBS groups increased water intake compared to the NaCl group, but did not lead to significant body weight gain. The BS and PBS groups increased water intake compared to the NaCl group, but did not lead to significant more body weight. BS and PBS might be able to induce one to drink much water and excrete much urine for extra sodium secretion. It needs further studies to conform this possible hypothesis. In conclusion, we demonstrated that BS regulated blood pressure through the WNK4 and

NCC-independent signal pathway.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

N/A

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