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Research Article

Effects of Roasting and Extrusion Puffing on the Antihypertensive Activity of Blended Grains in Spontaneously Hypertensive Rats

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We investigated the effects of processing (roasting and extrusion puffing) on the antihypertensive activity of blended grains both *in vitro* and *in vivo*. The blended grains were composed of sorghum (*Sorghum bicolor*), adzuki bean (*Vigna angularis*), and finger millet (*Eleusine coracana*). In the *in vitro* studies, total phenolic content (TPC), total flavonoid content (TFC), and angiotensin-converting enzyme (ACE) inhibitory activity of blended grains were evaluated. The results showed a significant decreasing trend in TPC, TFC, and ACE inhibitory activity in the following order: nonprocessed blended grains (NPBG) > roasted blended grains (RBG) > extrusion puffed blended grains (EPBG). Spontaneously hypertensive rats (SHRs) were fed a diet containing 40% blended grains (replacing the standard diet) for 8 weeks. None of the blended grains had any effects on feed intake, liver function, and serum lipid profile. Notably, NPBG showed the greatest reduction in systolic blood pressure compared to the model control (MC), followed by RBG, and EPBG, whereas diastolic blood pressure was significantly decreased in NPBG and RBG. Serum ACE activity and angiotensin II levels significantly decreased in all blended grains, with NPBG and RBG showing the most significant decrease in angiotensin II levels. Moreover, the mRNA expression of renin, a hormone involved in hypertension, was significantly decreased in NPBG and RBG. Only NPBG ameliorated aortic vascular remodeling compared to the MC. These results suggest that NPBG exhibited the most potent antihypertensive activity; however, roasting could be an effective processing method for maintaining antihypertensive effects compared to extrusion puffing.

1. Introduction

Hypertension is a severe disease with a high mortality rate worldwide that can lead to complications such as heart failure, arteriosclerosis, and stroke [1, 2]. Because essential hypertension accounts for over 80% of all hypertensive cases and occurs without any clear cause, research has been conducted to improve essential hypertension [3, 4]. Drugs such as captopril and ramipril are used to treat hypertension; however, they have been reported having side effects, including edema and dizziness [5]. Consequently, there is an increasing interest in exploring natural foods for the treatment of hypertension [6].

Grains, one of the most consumed foods worldwide, may help alleviate cardiovascular disease and metabolic syndrome owing to their high phytochemical content, including polyphenol compounds, flavonoids, and tannins [7, 8]. Sorghum (*Sorghum bicolor*) is abundant in various phenolic compounds, particularly 3-deoxyanthocyanidins and tannins, which can potentially reduce the risk of cardiovascular diseases [9, 10]. Adzuki bean (*Vigna angularis*) has been reported to be associated with the improvement of vascular oxidative stress and inflammation owing to its rich content of phytochemicals, particularly catechins, procyanidins, and saponins [11, 12]. Finger millet (*Eleusine coracana*), a type of millet, is effective in maintaining blood pressure due to its

elevated levels of polyphenols such as ferulic acid and proanthocyanidins, as well as its higher calcium content than other millet varieties [13, 14]. Accordingly, the antihypertensive effects of various grains, including sorghum, adzuki bean, and finger millet, have been extensively investigated.

Grains are usually processed to enhance digestibility and eliminate antinutritional factors [15–17]. Processing methods such as roasting and extrusion puffing are widely used in grain-based food production. Roasting involves a high-heat process for a short period of time, resulting in a crispy texture and enhanced flavor [18]. Extrusion puffing, however, utilizes gas pressure and thermal phase changes, which thereby increases volume and improves digestibility [19, 20]. As such, processing improves various aspects of grains; however, several studies have reported that thermal processing can result in a reduction in the bioactive compound content of the grains, thereby losing their potential health benefits [21, 22]. Thus, research on processing methods that can minimize the reduction in the antihypertensive activity of grains is necessary.

Nevertheless, research on processing methods without losing their biological activity of grains is limited, and comparative studies on the antihypertensive effects of roasting and extrusion puffing have not been conducted. Therefore, this study aimed to investigate the antihypertensive effects of blended grains to identify a processing method that preserves their antihypertensive activity.

2. Materials and Methods

2.1. Sample Preparation. Sorghum (*Sorghum bicolor*, Sodamchal), adzuki bean (*Vigna angularis*, Arari), and finger millet (*Eleusine coracana*, Finger1ho) were grown in 2021 and provided by the National Institute of Crop Science (Rural Development Administration, Suwon, Korea). After being fully ground, sorghum, adzuki bean, and finger millet were blended in a ratio of 35 : 35 : 30. The blending ratio was determined based on preliminary experiments evaluating the antihypertensive effects at various mixing ratios. Roasting was conducted at 220°C for 5 min, and extrusion puffing was performed using a Welly snack machine S3 model (Jain Inc., Bucheon, Korea) at 120°C and 120 rpm.

2.2. Determination of TPC and TFC in Blended Grain Extracts. The total phenolic content (TPC) and total flavonoid content (TFC) of each sample were measured as follows: samples were extracted with 80% ethanol at a ratio of 1 : 10 (w/v) and concentrated using a rotary evaporator (PuSHB-3S, Daihan Scientific, Wonju, Korea). TPC was determined using a method described by Xiang et al. [23], with some modifications. In brief, 200 μL of the sample was mixed with 500 μL of 0.2 N Folin–Ciocalteu reagent and allowed to react for 5 min. Then, 500 μL of 7.5% (w/v) Na_2CO_3 was added, and the mixture was allowed to react for another at 50°C 10 min. Subsequently, the absorbance at 760 nm was determined with a microplate reader (Bio Tek, Winooski, VT, USA). Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard to quantify TPC. TFC was determined using a method described by Xiang et al. [23], with some modifications. In brief, 300 μL of the sample was mixed with 300 μL of a 2% (w/v) aluminum chloride solution and allowed to react at room temperature for 10 min. Then, the absorbance at 415 nm was determined with a microplate reader (Bio Tek). Quercetin (Sigma-Aldrich) was used as the standard to quantify TFC.

2.3. Analysis of the ACE Inhibitory Activity in Blended Grain Extracts. An angiotensin-converting enzyme (ACE) inhibition assay was performed by slightly modifying the method described by Cushman and Cheung [24]. The amount of hippuric acid generated by the reaction of the substrate, hippuryl-His-Leu, with ACE was measured. The ACE solution was extracted from lung acetone powder from rabbit (Sigma-Aldrich). The substrate solution was prepared by dissolving hippuryl-His-Leu acetate salt (Sigma-Aldrich) in 0.1 M sodium borate buffer. The substrate solution (100 μL) was mixed with the sample (50 μL) and reacted at 37°C for 10 min. ACE solution (50 μL) was added and the reaction was conducted at 37°C for 60 min. The reaction was stopped by adding 250 μL of 1 N HCl. The mixture was then partitioned with 1 mL of ethyl acetate (Samchun Chemicals, Seoul, Korea). The supernatant (800 μL) was collected and dried. Next, the dried residue was dissolved in 1 mL of 0.1 M sodium borate buffer, and the absorbance thereof was measured at 228 nm using a spectrophotometer (GEN10S UV-Vis, Thermo Fisher Scientific, Waltham, MA, USA). The ACE inhibitory activity was calculated using the following formula:

$$\text{ACE inhibitory activity } (\%) = \left[1 - \frac{absorbance\ of\ sample - absorbance\ of\ sample\ blank}{absorbance\ of\ control - absorbance\ of\ control\ blank}\right] \times 100. \quad (1)$$

2.4. Animals and Diets. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Hanyang University (Seoul, Korea) (approval number: HY-IACUC-22-0055) and conducted according to the ethical regulations of the IACUC. Six-week-old male Wistar–Kyoto rats (WKY) and spontaneously hypertensive rats (SHRs) were obtained from Central Lab Animal Inc. (Seoul, Korea) and acclimatized for 1 week. Rats were randomly divided into five experimental groups ($n = 6$): (1) WKY fed with the standard diet (NC, normal control); (2) SHR fed with the standard diet (MC, model control); (3) SHR fed with a diet of 40% (w/w)

nonprocessed blended grains (NPBG); (4) SHR fed with a diet of 40% (w/w) roasted blended grains (RBG); and (5) SHR fed with a diet of 40% (w/w) extrusion puffed blended grains (EPBG). The room temperature was maintained at 22 ± 1°C, and the relative humidity was maintained at 50 ± 10%, with a 12 h light/dark cycle. All the rats were allowed free access to food and water throughout the experiment. The experimental diets were prepared by substituting 40% (w/w) of the AIN-93G diet (Doo Yeol Biotech, Seoul, Korea) with the blended grain, which was stored at −20°C until use. The experimental diets were provided in pellet form and their compositions are listed in Table 1. During the experimental period, body weight was measured once per week and feed intake was measured twice a week. After the experiment was completed, the animals were fasted for 12 h and then anesthetized via an intraperitoneal injection of xylazine (15 mg/kg BW) and ketamine (100 mg/kg BW). Blood was collected via cardiac puncture, and the kidneys and aortas were recovered via laparotomy. The blood and organs were stored at −80°C until analysis.

Table 1: Composition of experimental diets (g/kg).

	AIN-93G	NPBG	RBG	EPBG
Casein	200	141.1	137.3	135.4
L-Cystine	3	3	3	3
Corn starch	397	109.3	99.3	95.8
Maltodextrin	132	132	132	132
Sucrose	100	100	100	100
Cellulose	50	18.1	18.7	17.1
Soybean oil	70	63.2	63.3	67.0
Mineral mix S10022G	35	35	35	35
Vitamin mix V10037	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5
NPBG		400		
RBG			400	
EPBG				400

NPBG, nonprocessed blended grains; RBG, roasted blended grains; EPBG, extrusion puffed blended grains.

2.5. Growth Performance Analysis. During the experimental period, body weight was measured once a week and feed intake was measured twice a week for 8 weeks to investigate the effects of blended grain intake on body weight, feed intake, and feed efficiency ratio (FER). The FER was calculated by dividing the weight gain during the experiment by the amount of feed intake during the same period.

2.6. Blood Pressure Measurement. During the experimental period, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured once a week for 8 weeks using the tail-cuff method. The rats were placed in a BP-2000 holder (Visitech Systems, Apex, NC, USA) and allowed to acclimate for 5 min in a heating chamber at 36°C before the tail-cuff was attached to measure blood pressure. To minimize measurement errors, the blood pressure of each rat was measured at least three times.

2.7. Blood Biochemical Analysis. Blood was collected via cardiac puncture, placed in tubes containing ethylenediaminetetraacetic acid (EDTA), and centrifuged to separate the plasma. Serum levels of total cholesterol (TC), triglycerides (TG), aspartate aminotransferase (AST), and alanine transaminase (ALT) were measured using commercial kits (Asan Pharmaceutical, Seoul, Korea). Serum ACE activity was measured using the method described by Schwager et al. [25], with some modifications. In brief, 3 μL of serum was mixed with 30 μL of 5.7 mM hippuryl-His-Leu (Sigma-Aldrich) and incubated at 37°C for 25 min. Then, 117 μL of 0.28 M NaOH (Sigma-Aldrich) and 45 μL of 20 mg/mL o-phthaldialdehyde (Sigma-Aldrich) were added, and the mixture was incubated at room temperature for 10 min. The reaction was stopped by adding 75 μL of 3 M HCl (Sigma-Aldrich), and the upper layer was extracted after centrifugation. The fluorescence intensity was measured at excitation and emission wavelengths of 360 and 460 nm, respectively. Serum angiotensin II levels were measured using an angiotensin II ELISA kit (RayBiotech, Inc., Norcross, GA, USA).

2.8. Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR). To extract RNA from the renal tissue, the kidneys of experimental rats were homogenized using TRIzol (Ambion, Austin, TX, USA), and RNA was isolated using the PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA, USA). RNA concentration was measured using a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA). Purified RNA was reverse-transcribed into cDNA using the Prime Script™ RT reagent kit (Takara, Shiga, Japan). Real-time RT-PCR was performed using a CFX96TM RT-PCR detection system (Bio-Rad, Hercules, CA, USA), and SYBR Green was used to measure renin mRNA expression levels. For gene analysis, renin primer (sense: 5′-TGCTAAAGGAGGAAGTGTTT-3′; antisense: 5′-TGA TGCTCACGTAGTGAAAG-3′) was used, and the relative gene expression of renin was measured using GAPDH primer (sense: 5′-GTCGGTGTGAACGGATTTG-3′; antisense: 5′-TCCCATTCTCAGCCTTGAC-3′).

2.9. Histological Analysis of the Aorta. On the day of autopsy, the recovered aortas were fixed in 10% neutral buffered formalin (Sigma-Aldrich), dehydrated, and embedded in paraffin to prepare tissue sections. The sections were stained with hematoxylin and eosin (H&E) to measure the intima-media thickness (IMT) and lumen diameter (LD) of the blood vessel. The IMT and LD of the aorta were measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.10. Statistical Analysis. All statistical analyses were performed using the GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA). Results are expressed as the mean ± standard error of the mean (SEM). One-way ANOVA was performed for comparisons among multiple

groups, followed by Tukey's post hoc test. When the p value was less than 0.05 ($p < 0.05$), the results were considered statistically significant.

3. Results and Discussion

3.1. TPC, TFC, and ACE Inhibitory Activities. The TPC, TFC, and ACE inhibitory activities of the blended grains are shown in Figure 1. TPC and TFC were significantly lower in both RBG and EPBG than in NPBG ($p < 0.05$). Among the processed blended grain groups, RBG showed significantly higher TPC and TFC than EPBG ($p < 0.05$). These findings are consistent with those of a previous study that reported a decrease in phenolic compounds in processed grains [26]. The reduction in TPC and TFC can be attributed to the high temperature during heating, which causes the oxidative breakdown of phenolic compounds in grains, such as sorghum or adzuki bean [27, 28]. Similarly, heat processes, such as roasting and boiling, can diminish the antioxidant activity of finger millet via oxidation and decomposition reactions [29]. These results suggested that thermal processing, including roasting and extrusion puffing, can negatively affect the TPC and TFC of grains.

However, it is worth noting that roasting may retain higher TPC and TFC than extrusion puffing. A previous study reported that roasting could preserve the antioxidant activity by generating Maillard reaction products [30]. In addition, roasting can damage the cellular structure of cereal grains, leading to the release of bound phenolic compounds [31]. Furthermore, the thermal decomposition of protein-lipid complexes that occurs during roasting may be associated with an increase in carotenoid content [32]. Based on these studies, it can be inferred that roasting might more greatly mitigate the decrease of TPC and TFC than extrusion puffing.

ACE inhibitory activity was significantly lower in RBG and EPBG than in NPBG ($p < 0.05$). In addition, EPBG significantly decreased ACE inhibitory activity compared to RBG ($p < 0.05$). The decrease in the ACE inhibitory activity can be attributed to a reduction in TPC and TFC during processing. Phenolic compounds can suppress ACE by competing with its active site [33]. Similarly, a previous study showed that the ACE inhibitory activity of whole rice was significantly reduced by processing because of the decrease in phenolic compounds [34]. In conclusion, roasting and extrusion puffing decreased the content of bioactive compounds and ACE inhibitory activity in blended grains; however, roasting may better retain bioactive compounds and ACE inhibitory activity than extrusion puffing.

3.2. Growth Performance. The growth performances of the experimental animals are shown in Table 2. Body weights were significantly lower in all SHR experimental groups (MC, NPBG, RBG, and EPBG) than in the NC group ($p < 0.05$). These results are consistent with those of previous studies showing that SHRs have a lower weight gain rate than WKY due to genetic differences [35, 36]. Notably, the final body weight of the NPBG rats was significantly higher than that of the MC, RBG, and EPBG rats ($p < 0.05$). These findings suggest that NPBG may help promote growth by overcoming the weight gain resistance attributed to hypertension-related genes. In addition, FER was significantly higher in the NC group than in the MC, NPBG, RBG, and EPBG groups ($p < 0.05$). However, there were no significant differences in feed intake and FER among the MC, NPBG, RBG, and EPBG groups. In summary, the effects of weight gain were observed only in the NPBG group, whereas the consumption of blended grains did not affect feed intake and FER in SHR.

3.3. Systolic Blood Pressure and Diastolic Blood Pressure. Changes in SBP and DBP in the experimental animals are shown in Figure 2. Initially, all SHRs exhibited elevated SBP and DBP, confirming the successful establishment of essential hypertension. Throughout the experimental period, the NC maintained a normal range of blood pressure, whereas the MC showed a progressive increase in blood pressure. Starting from the third week of the experiment, SBP in the NPBG, RBG, and EPBG groups was significantly lower than that in the MC group ($p < 0.05$). In addition, SBP in the final week was significantly decreased in all blended grain groups compared to MC ($p < 0.05$); NPBG exhibited the greatest reduction, followed by RBG, and then EPBG. As for DBP in the final week, only the NPBG and RBG showed a significant decrease compared with the MC ($p < 0.05$), whereas the reduction observed in the EPBG was not statistically significant.

The blood pressure-lowering effects of blended grains, including adzuki bean, finger millet, and sorghum, are supported by the results of previous studies. Adzuki bean and finger millet extract alleviated blood pressure by regulating the renin-angiotensin system or inhibiting inflammatory cytokines [37–39]. In addition, sorghum consumption reduces blood pressure and protects against oxidative stress-induced vascular damage [40]. More importantly, this study revealed that roasting had superior blood pressure-lowering effects compared to extrusion puffing. Our *in vitro* investigations found that the TPC and TFC of the blended grains decreased after processing, which may correlate with the blood pressure results. A previous study has shown that polyphenols, which are abundant in the bran of grains, can reduce blood pressure in spontaneously hypertensive rats via nitric oxide-mediated vasodilation [41]. In addition, dietary flavonoids exert blood pressure-lowering effects in spontaneously hypertensive rats by improving endothelial function [42].

Thus, roasting, which exhibits a lesser reduction in bioactive compounds than extrusion puffing, can be an effective processing method for maintaining antihypertensive activity. Considering that most grains undergo processing before consumption, it is essential to highlight the potential of roasting as a promising processing for preserving the antihypertensive properties of blended grains.

3.4. Blood Biochemical Analysis. The AST, ALT, TG, and TC levels in the experimental animals are presented in Table 3. AST and ALT are liver enzymes that increase with liver

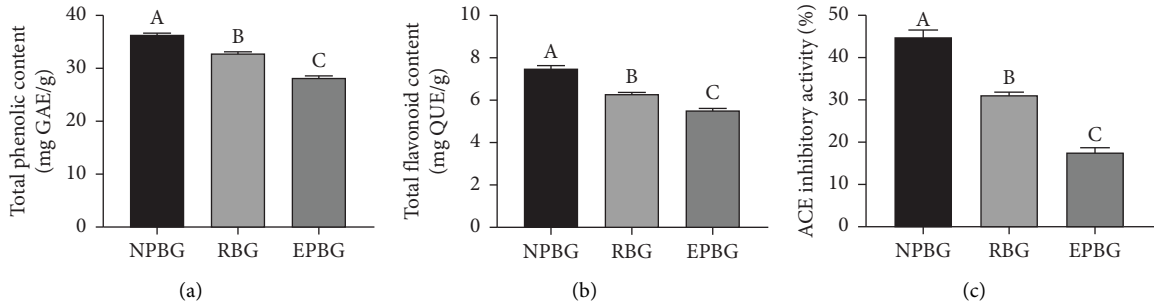

Figure 1: (a) Total phenolic content, (b) total flavonoid content, and (c) *in vitro* ACE inhibitory activity of differently processed blended grains. GAE, gallic acid equivalent; QUE, quercetin equivalent; ACE, angiotensin-converting enzyme; NPBG, nonprocessed blended grains; RBG, roasted blended grains; EPBG, extrusion puffed blended grains. Data are expressed as the means ± standard error of the means. $^{(A–C)}$The values with different letters indicate significant differences at $p < 0.05$.

Table 2: Effects of differently processed blended grains on growth performance in rats for 8 weeks.

	NC	MC	NPBG	RBG	EPBG
Body weight (g)	393 ± 2.17^{a}	341 ± 3.19^{c}	364 ± 1.43^{b}	349 ± 4.09^{c}	350 ± 2.32^{c}
Feed intake (g/day)	17.1 ± 0.09^{b}	17.9 ± 0.30^{ab}	18.3 ± 0.27^{ab}	18.9 ± 0.32^{a}	17.9 ± 0.08^{ab}
FER (%)	19.1 ± 0.26^{a}	14.0 ± 0.27^{b}	14.2 ± 0.12^{b}	13.9 ± 0.52^{b}	13.8 ± 0.34^{b}

NC, normal control; MC, model control; NPBG, nonprocessed blended grains; RBG, roasted blended grains; EPBG, extrusion puffed blended grains; FER, feed efficiency ratio. Data are expressed as the means ± standard error of the means. $^{(a–c)}$Values with different letters within the same row are significantly different at $p < 0.05$.

damage and are indicators of hepatic toxicity [43, 44]. Serum AST levels were not significantly different among all experimental groups; however, serum ALT levels were significantly increased in MC compared with NC ($p < 0.05$), and there were no significant differences in NPBG, RBG, and EPBG compared with MC. These results are consistent with a previous study that reported that higher levels of ALT in hypertension are associated with the inflammatory response in hypertension [45]. Importantly, the AST and ALT levels of all experimental groups were within the normal range (<40 IU/L), suggesting that there was no hepatotoxicity from consuming the blended grains.

Serum TG levels were not significantly different among all experimental groups; however, serum TC levels were significantly increased in the NC group compared to all SHR experimental groups ($p < 0.05$). However, there was no significant difference in TC levels among the SHR experimental groups. In summary, serum TG and TC levels did not exhibit significant differences among all SHR experimental groups, indicating that the consumption of blended grains did not affect serum lipid profiles.

3.5. ACE Activity and the Angiotensin II Level in Serum and mRNA Expression of Renin in the Kidney. The renin-angiotensin system plays a crucial role in regulating blood pressure. ACE converts angiotensin I to angiotensin II, resulting in vasoconstriction and an increased blood pressure [46, 47]. A previous study showed that decreased ACE activity is associated with reduced systolic blood pressure in SHR [48]. Angiotensin II is generated by ACE via the removal of two C-terminal residues (His-Leu) from angiotensin I [25]. Renin is a hormone secreted by the kidneys that participates in aldosterone synthesis and angiotensin activation. Decreased renin mRNA expression is associated with improved hypertension [49].

In this study, serum ACE activity, angiotensin II levels, and renal mRNA expression of renin in rats were analyzed (Figure 3). MC exhibited higher ACE activity, angiotensin II levels, and renin mRNA expression in the kidney than NC ($p < 0.05$). These results are consistent with those of a previous study showing that SHRs have enhanced activity within the renin-angiotensin system associated with hypertension [50]. Notably, NPBG, RBG, and EPBG significantly reduced the serum ACE activity compared to that in the MC group ($p < 0.05$). This can be attributed to the ACE inhibitory potentials of grains enriched in polyphenols, which bind to the ACE active site [51, 52]. In addition, all blended grain groups showed significantly decreased angiotensin II levels compared with the MC group ($p < 0.05$). Specifically, angiotensin II levels were significantly lower in the NPBG and RBG than in the EPBG ($p < 0.05$). Furthermore, the renin mRNA expression was significantly decreased in the NPBG and RBG compared with that in the MC ($p < 0.05$).

Interestingly, NPBG and RBG showed a higher ability to modulate the renin-angiotensin system than EPBG, which can be attributed to the impact of processing on polyphenol content and bioavailability. A previous study demonstrated the potential loss of bioactive constituents during extrusion puffing [53, 54]. In addition, the low effectiveness of EPBG in regulating the renin-angiotensin system was supported by *in vitro* experiments that demonstrated low ACE inhibitory activity of EPBG. Overall, all the blended grains regulated the renin-angiotensin system, which is the main mechanism of hypertension, with NPBG and RBG exhibiting greater modulatory effects than EPBG.

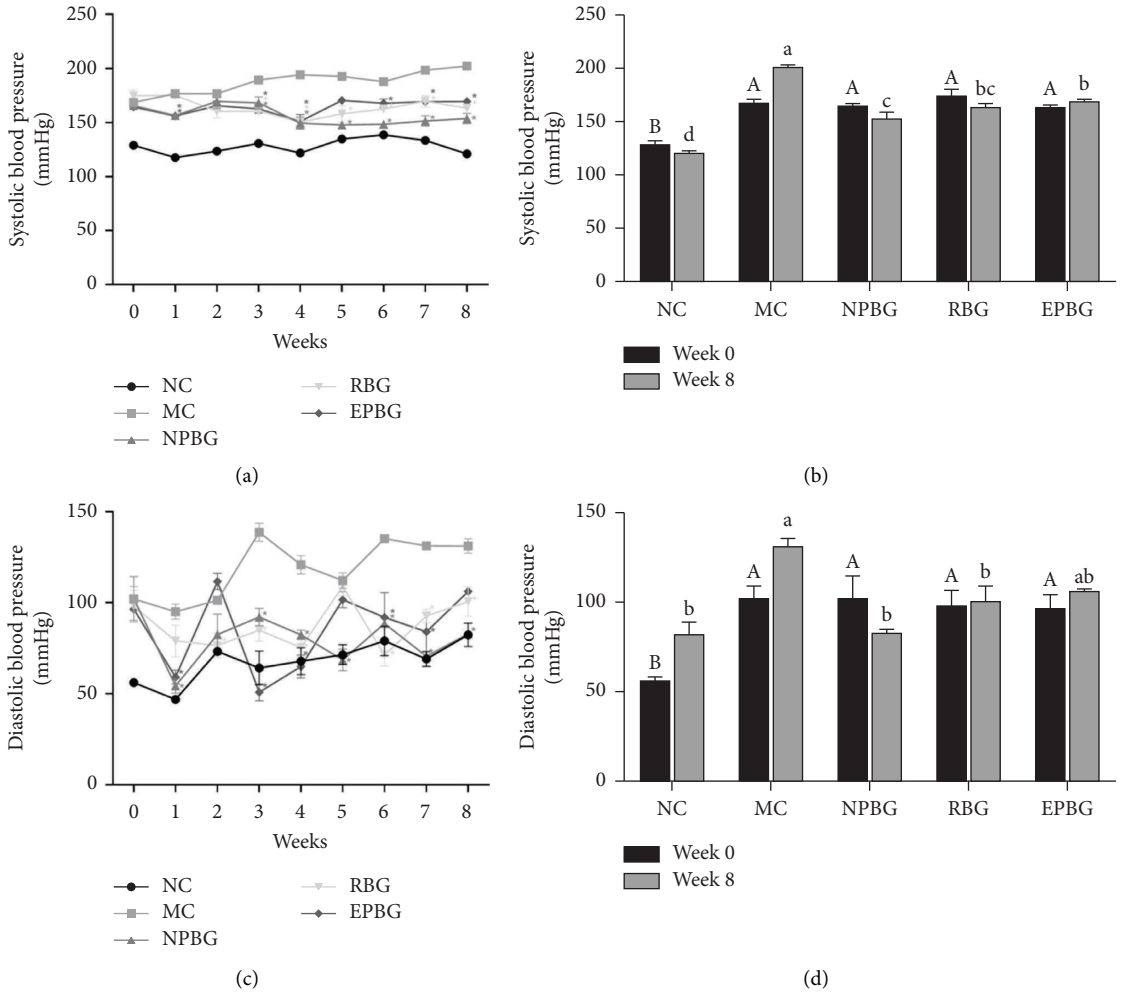

Figure 2: (a) Weekly systolic blood pressure, (b) systolic blood pressure at week 0 and week 8, (c) weekly diastolic blood pressure, and (d) diastolic blood pressure at week 0 and week 8 in the rats supplemented with differently processed blended grains for 8 weeks. NC, normal control; MC, model control; NPBG, nonprocessed blended grains; RBG, roasted blended grains; EPBG, extrusion puffed blended grains. Data are expressed as the means ± standard error of the means. *Significant differences from MC at $p < 0.05$. $^{(A\text{-}B)}$The difference in systolic blood pressure at week 0 indicates significant differences at $p < 0.05$. $^{(a-d)}$The difference in systolic blood pressure at week 8 indicates significant differences at $p < 0.05$.

Table 3: Effects of differently processed blended grains on blood biochemical parameters in the serum of the rats for 8 weeks.

	NC	MC	NPBG	RBG	EPBG
AST (IU/L)	16.6 ± 1.12^{a}	18.0 ± 0.52^{a}	17.7 ± 1.80^{a}	14.3 ± 1.08^{a}	15.8 ± 0.60^{a}
ALT (IU/L)	3.25 ± 0.20^{c}	12.8 ± 1.38^{ab}	17.0 ± 2.24^{a}	11.2 ± 0.86^{ab}	7.45 ± 0.26^{bc}
TG (mg/dL)	122 ± 0.91^{a}	121 ± 2.13^{a}	117 ± 1.56^{a}	119 ± 0.98^{a}	123 ± 3.69^{a}
TC (mg/dL)	91.0 ± 0.93^{a}	66.5 ± 2.69^{b}	57.2 ± 4.12^{b}	57.5 ± 0.47^{b}	59.0 ± 1.23^{b}

AST, aspartate aminotransferase; ALT, alanine transaminase; TG, triglyceride; TC, total cholesterol; NC, normal control; MC, model control; NPBG, nonprocessed blended grains; RBG, roasted blended grains; EPBG, extrusion puffed blended grains. Data are expressed as the means ± standard error of the means. $^{(a-c)}$Values with different letters within the same row are significantly different at $p < 0.05$.

3.6. Histological Analysis of the Aorta. Histological changes in the aorta are illustrated in Figure 4. The IMT and ratio of IMT to LD of the aorta significantly increased in the MC group compared to those in the NC group ($p < 0.05$), indicating elevated vascular resistance. The difference in vascular thickness between WKY and SHR is consistent with a previous study that SHR are stimulated by angiotensin, leading to thicker vascular walls [55]. Notably, NPBG significantly decreased IMT and the ratio of IMT to LD of the aorta compared to MC ($p < 0.05$), whereas RBG and EPBG showed no significant differences compared to MC. However, there was no significant difference in the LD of the blood vessels among the experimental groups.

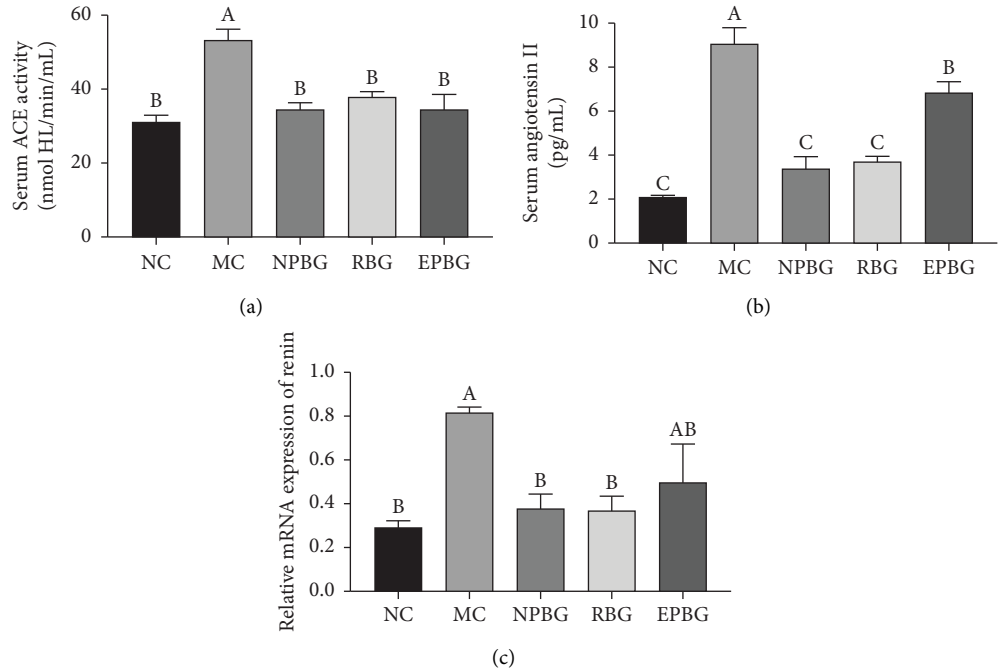

Figure 3: (a) ACE activity in the serum, (b) angiotensin II levels in the serum, and (c) mRNA expression of renin in the kidney of the rats supplemented with differently processed blended grains for 8 weeks. ACE, angiotensin-converting enzyme; NC, normal control; MC, model control; NPBG, nonprocessed blended grains; RBG, roasted blended grains; EPBG, extrusion puffed blended grains. Data are expressed as the means ± standard error of the means. $^{(A–C)}$The values with different letters indicate significant differences at $p < 0.05$.

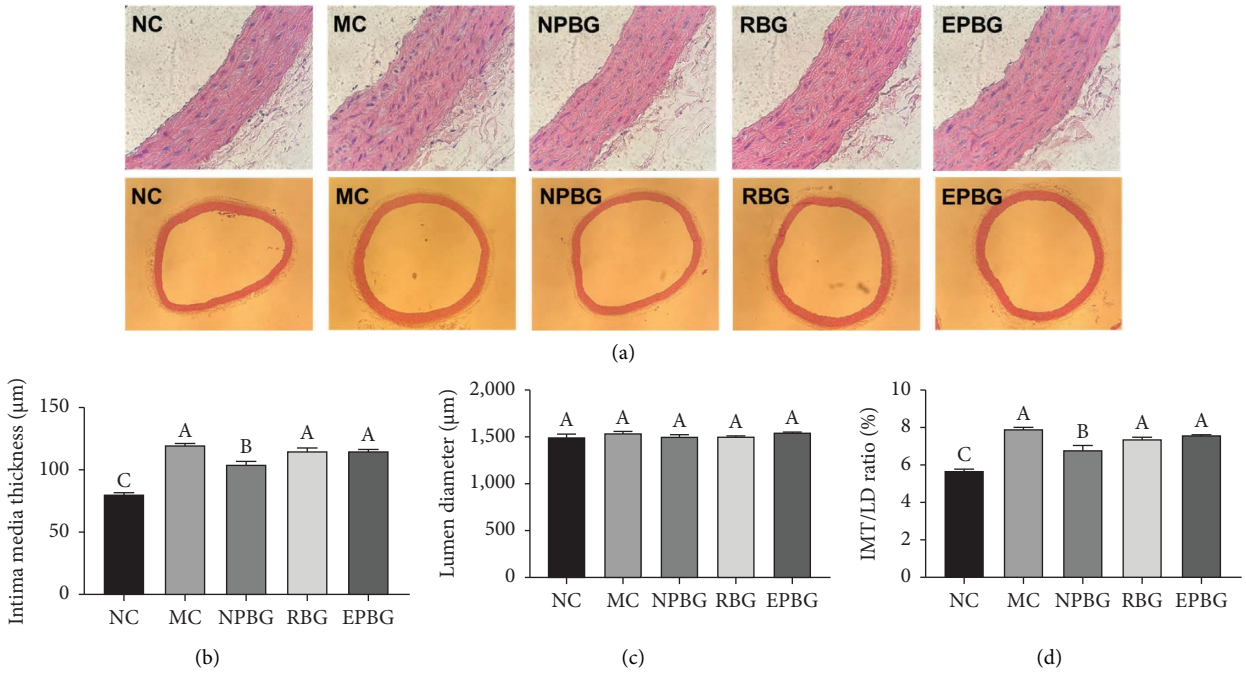

Figure 4: (a) Histopathological changes in the aorta were observed via hematoxylin and eosin staining ×40 and ×10, (b) intima-media thickness (IMT), (c) lumen diameter (LD), and (d) IMT/LD of the aorta. NC, normal control; MC, model control; NPBG, nonprocessed blended grains; RBG, roasted blended grains; EPBG, extrusion puffed blended grains. Data are expressed as the means ± standard error of the means. $^{(A–C)}$The values with different letters indicate significant differences at $p < 0.05$.

While previous studies have demonstrated the positive effect of grains on vascular remodeling, this is the first study to investigate the effects of differently processed grains on the aorta. A previous study demonstrated that the ACE inhibitory activity plays a significant role in ameliorating vascular remodeling by suppressing vascular constriction [56]. In line with these findings, NPBG showed a remarkable improvement in vascular remodeling of the aorta, which may be attributed to its ACE inhibitory activity. However, RBG and EPBG, which demonstrated ACE inhibitory effects, did not improve vascular remodeling in the aorta. Therefore, the NPBG has the greatest potential to mitigate vascular remodeling compared with the RBG and EPBG. Further research is required to explore processing methods beyond roasting and extrusion puffing, which may have a positive impact on vascular remodeling.

4. Conclusions

In this study, the intake of all blended grains dramatically reduced SBP in SHR, and the effects of reduction are as follows: NPBG > RBG > EPBG. In addition, NPBG and RBG significantly lowered DBP. Furthermore, all blended grains resulted in a considerable reduction in serum ACE activity and angiotensin II levels, with NPBG and RBG showing the most significant reduction in angiotensin II levels. NPBG and RBG also significantly decreased renin mRNA expression in SHR, and NPBG remarkably improved aortic vascular remodeling. These findings indicated that the antihypertensive effects of the blended grains aligned with the trend observed *in vitro*. Considering that most grains undergo processing before consumption, roasting could be an effective method to minimize the reduction in the antihypertensive properties of blended grains. Therefore, roasting can be used to develop processed products from blended grains.

Data Availability

The data will be made available from the corresponding author on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Byungkwon Han conceptualized the study, performed data curation, visualization, and formal analysis, prepared the original draft, and reviewed and edited the manuscript. Eun Woo Jeong performed data curation, prepared the original draft, and reviewed and edited the manuscript. Areum Han and Youjin Baek validated the data and reviewed and edited the manuscript. Hyun-Joo Kim conceptualized the study and reviewed and edited the manuscript and was responsible for resources. Hyeon Gyu Lee reviewed and edited the manuscript and was responsible for funding acquisition, resources, and validation.

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