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Effects of Different Types of Yam (*Dioscorea alata*) Products on the Blood Pressure of Spontaneously Hypertensive Rats

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The aim of the present study was to investigate different yam treatments, including powdered-yam-products (PYP) and liquid-yam-products (LYP), with respect to spontaneously hypertensive rats (SHRs) blood pressure. PYP included alcohol-insoluble-solids of yam tuber, hot-air-drying (HAD) of yam tuber slices, steam-cooked once or twice followed by HAD which were subsequently powdered. LYP included water extracts of yam tuber (WEY) heated at 90 °C (WEY90H) or 95 °C for 10 min (WEY95H), and then stored at 4 °C for different numbers of day. PYP, WEY, and WEYH were found effectively to reduce the blood pressure of SHR and should be beneficial in food processing in the development of functional foods for blood pressure regulation.

Key words: antihypertensive activity; blood pressure; spontaneously hypertensive rat (SHR); yam

Several classes of pharmacological agents are used in the treatment of hypertension. One class of anti-hypertensive drugs, known as angiotensin converting enzyme (ACE) inhibitors, is associated with a low rate of adverse side-effects, and it is the preferred class of anti-hypertensive agents for treating patients with concurrent secondary diseases.[1)] Several food-derived peptides have been reported to inhibit ACE,[2)] including α-lactalbumin and β-lactoglobulin,[3,4)] casein,[5,6)] and zein,[7,8)] all of which are hydrolyzed by pepsin, trypsin, or chymotrypsin. Fujita *et al.*[9)] found that the octapeptides of FFGRCVSP ($IC_{50} = 0.4\,\mu M$) and ERKIKVYL ($IC_{50} = 1.2\,\mu M$) are potent ACE inhibitors, but none of them was effective in animal models to reduce the blood pressure of SHRs. Sato *et al.*[10)] pointed out that three dipeptides, AW ($IC_{50} = 18.8\,\mu M$), VW ($IC_{50} = 3.3\,\mu M$), and LW ($IC_{50} = 23.6\,\mu M$), were potential ACE inhibitory peptides, but, none of them were able to reduce the blood pressure of SHRs in animal models effectively.

We have reported that the purified storage protein, dioscorin, from yam tubers exhibited ACE inhibitory activity,[11)] antihypertensive effects on SHRs,[12)] antioxidant activity,[13,14)] and immunomodulatory activity.[15)] The results of our recent feeding trial revealed that instant food containing lyophilized yam powders had regulating effects on human blood pressure.[16)] Dried slices of yam tuber are frequently used as Chinese herbal medicines, and the fresh tuber is also a staple food in West Africa, South Asia, the Caribbean, and Taiwan. In the present study, different yam products, powdered yam products (PYP) and liquid yam products (LYP), were prepared to examine their health benefits and to investigate the antihypertensive activity they might have on SHR. It was found that PYP and LYP had antihypertensive activities toward SHRs. This might be useful information for food processing in the development of functional foods for blood-pressure regulation.

Materials and Methods

Materials. DE-52 anion exchange resin was from Whatman (Florham Park, NJ); bovine serum albumin, Coomassie Brilliant Blue R-250, Tris–HCl, and other chemicals and reagents were from Sigma Chemical (St. Louis, MO).

Purification of the yam tuber storage protein dioscorin. Fresh yam (*Dioscorea alata* L.) tuber was purchased from a local wholesaler (Taipei, Taiwan). After being washed and peeled, the tubers were cut into pieces for storage protein purification, as described in previous reports.[11–15,17,18)] The yam tubers were homogenized with 4 volumes (W/V) of 50 mM Tris–HCl buffer (pH 8.3). After centrifugation at 12,500 *g* for 30 min, the supernatants were saved and loaded directly onto a DE-52 ion exchange column. After being washed with three column volumes of 50 mM Tris–HCl buffer (pH 8.3), the adsorbed dioscorin was eluted batchwise with the same washing buffer containing 150 mM NaCl. The eluted fraction was collected and concentrated with Ultrafree-4 (molecular weight cutoff, 5 kDa, Millipore, Bedford, MA). The concentrated dioscorin solution was dialyzed against deionized water overnight and then lyophilized. The storage protein of dioscorin was used to plot the standard curve to quantify protein contents in the water extracts of the yam tuber using a Bio-Rad protein assay kit (Hercules, CA).[19)]

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Abbreviations: ACE, angiotensin converting enzyme; AIS, alcoholic-insoluble-solids; HAD, hot-air-drying; LYP, liquid-yam-products; PYP, powdered-yam-products; SCO, steam-cooked once; SCT, steam-cooked twice; SHR, spontaneously hypertensive rat; WEY, water extracts of yam tuber; WEY90H, WEY heated at 90 °C for 10 min; WEY95H, WEY heated at 95 °C for 10 min

Preparation of the alcohol-insoluble-solids (AIS) from the yam tuber. The AIS of yam tuber was prepared according to a previous method.[20,21] In brief, strips of peeled yam tuber were homogenized in a blender twice with absolute ethanol for 1-min periods to a final 80% concentration. The homogenates were kept at 60 °C in a water bath for at least 40 min before being cooled to room temperature. The heated homogenates were filtered through Whatman no. 1 filter paper, and the residues were homogenized twice with 80% ethanol for 1-min periods. After they were refiltered, the residues were washed twice with 80% ethanol, rinsed with acetone, and finally dried at 40 °C. The dried residues were homogenized with a blender for further use. The recovery of yam AIS from fresh yam tuber was about 30%. The proximate compositions of processed yam powders were analyzed by AGV Products (Chiayi, Taiwan), which followed AOAC procedures.[22]

Steam-cooked once (SCO) and steam-cooked twice (SCT) yam tuber slices followed by hot-air drying (HAD). Each slice (about 0.5 cm in width) of peeled yam tuber was put on a steamer over an electric pot, and the cooking time was set for 40 min for the SCO yam tuber slices. After cooling, half of the SCO yam tuber slice was steam-cooked for another 40 min to become a SCT yam tuber slice. The SCO and SCT yam tuber slices together with the fresh yam tuber slices were put separately into a 50 °C oven as part of a hot-air drying (HAD) procedure for 2 d. The SCO-HAD, SCT-HAD, and HAD yam tuber slices were powdered with a blender for further use.

Preparation of liquid-yam-products (LYP). Peeled yam tubers were cut into pieces and homogenized with 2 volumes (W/V) of distilled water. After centrifugation at 12,500 *g* for 30 min, the supernatants were saved and adjusted to 3 volumes on a weight basis (W/V) with distilled water as water extracts of yam tuber (WEY), and stored at 4 °C for further use. For heating treatment, the WEY (in a 50-ml plastic tube) was put into a beaker immersed in a water bath heated to 90 °C for 10 min (WEY90H) or to 95 °C for 10 min (WEY95H) before being cooled immediately in an ice bath. For storage treatment, the heated and cooled WEY was stored at 4 °C for 1 d (WEY90H-S1, WEY95H-S1), 3 d (WEY90H-S3, WEY95H-S3), and 6 d (WEY90H-S6, WEY95H-S6) for antihypertensive activity assay.

SDS–PAGE gel electrophoresis and immunostaining. The AIS and HAD products were extracted with f4-fold volumes (W/V) of 100 mM Tris buffer (pH 8.3) overnight, and together with yam water extracts of different heat treatments were subjected to SDS–PAGE electrophoresis. Four volumes of sample were mixed with one volume of sample buffer (60 mM Tris–HCl buffer, pH 6.8, containing 2% SDS, 25% glycerol, and 0.1% bromophenol blue) heated at 100 °C for 5 min. After electrophoresis was finished, the gel was cut into two parts. One was fixed with 12.5% trichloroacetic acid for protein staining with Coomassie Brilliant Blue R-250; the other was equilibrated in Tris-glycine buffer (pH 8.3) and then transferred onto an Immobilon PVDF membrane (Millipore, Bedford, MA) for immunostaining detected by dioscorin polyclonal antibody.[15,23]

Antihypertensive effects of PYP and of LYP on SHR. The effects of orally-administered PYP-related and LYP-related products by feeding tube (2.0×80 mm) on the blood pressure of SHRs were determined.[12,16,24,25] All the animal experimental procedures followed published guidelines.[26] The male SHRs (8 weeks of age, National Laboratory Animal Center, Taipei, Taiwan) were housed individually in steel cages kept at 24 °C under a 12-h light-dark cycle, and had free acess to a standard mouse/rat chow (Prolab® RMH2500, 5P14 Diet, PMI Nutrition International, Brentwood, MO) and water. The SHRs were randomly divided into control and sample treatments for blood pressure determinations (six rats per group). For a short-term antihypertensive experiment, 0.5 ml of water-suspended PYP-related or 1 ml of LYP-related products was orally administered to the SHRs (AIS and HAD, 40 or 60 mg/kg of SHR; SCO-HAD and SCT-HAD, 80 mg/kg of SHR; WEY, WEYH, WEYH-S, 154.4 mg of protein/kg of SHR) once, and tail blood pressure was measured 4 times using an indirect blood pressure meter (BP-98A, Softron, Tokyo) for each treatment. For 30-d antihypertensive effects, WEY90H was orally administered to the SHRs once a day for 30-d (154.4 mg of protein/kg of SHR), and the blood pressure was measured once every 2 d before oral administration. Before blood pressure measurement, the SHRs were warmed for 10 min in a 39 °C thermostated box. Distilled water (0.5 or 1 ml) was used in a control experiment. Blood pressure was measured after oral administration of the distilled water. The means of triplicates were recorded. The measured blood pressure values were collected and averaged from six rats, termed $BP_{control}$. The measured blood pressure values of each rat after being administered treated sample orally were collected and averaged, termed the $BP_{treated\ sample}$. The six values calculated from $BP_{treated\ sample} - BP_{control}$ were averaged and are given as lowering effects in blood pressure changes (ΔBP)[9] in the treated samples at the same treatment time after oral administration (such as 2, 4, 6, 8, and 24 h) for short-term antihypertensive activity.

Statistical analysis. Means ± SD of triplicates were measured. Student's *t*-test was used for comparisons with control, and treatments were made at the same time interval or between WEY and WEYH or WEY and WEYH-S when $p < 0.05$ (*) or $p < 0.01$ (**) or $p < 0.001$ (***).

Results and Discussion

The proximate compositions of prepared PYPs, including AIS and HAD products, are shown in Table 1. The crude carbohydrate contained close to 80% in each preparation. The crude protein contents were 5.66 and 6.71% respectively for AIS and HAD of the yam products. The AIS and HAD products were extracted with 4-fold volumes (W/V) of 100 mM Tris buffer (pH 8.3) overnight, and SDS–PAGE electrophoresis was performed on the crude extracts for protein staining and then for immunostaining detected by dioscorin polyclonal antibody.[15,23] It was found that dioscorin was the main protein in the AIS and HAD extracts (arrow, Fig. 1A and C). The crude LYP was also determined for its protein contents using a Bio-Rad protein assay kit,[19] and bovine serum albumin or purified yam dioscorin was used as standard, but the proteins responded to very different slopes of the standard curve by the Bradford method.[19] Hence purified dioscorin, as a major yam tuber storage protein, was chosen to plot the protein standard curve. The calculated protein contents in the LYP were 154.4 mg of protein/ml. The LYP showed one main protein band (arrow, Fig. 1B) and other minor bands in SDS–PAGE gel by protein stains, and only one main protein band by immune stains was detected by dioscorin polyclonal antibody (Fig. 1D). It was clear that dioscorin was the main protein in LYP extracts. Heating treatment 90 °C for 10 min (WEY90H, Fig. 1B, lane 6) and 95 °C for 10 min (WEY95H, Fig. 1B, lane 7) did not significantly affect the amounts of dioscorin in comparison with the unheated treatment (Fig. 1B, lane 5). Our recent study[16] revealed that the lyophilized powders of

Table 1. Proximate Compositions of Prepared Powdered-Yam-Products (PYPs), Including Alcohol-Insoluble Solids (AIS) of Yam Tuber and Hot-Air-Drying (HAD) Yam Tuber Slices

	AIS (%)	HAD (%)
Protein	5.66	6.71
Fat	0.50	0.87
Carbohydrate	78.97	79.65
Ash	1.71	2.97
Moisture	13.16	9.80
Calorie (Kcal/100 g)	343.02	353.27

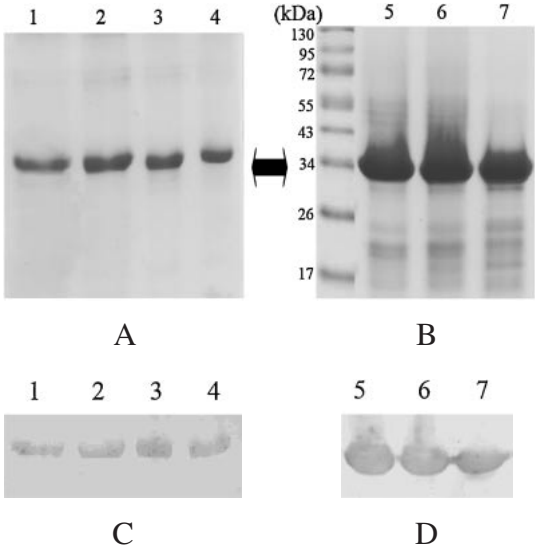

Fig. 1. The Extractable Protein Stains (A, B) and Immune Stains (C, D) of Different Yam Tuber Treatments, Including the Powdered-Yam-Products (A, C) and Liquid-Yam-Products (B, D).

After electrophoresis, 12.5% SDS–PAGE gel was stained with Comassie Brilliant Blue R-250 or blotted onto a PVDF membrane for immune stains by anti-dioscorin polyclonal antibody (from rabbit). Lanes 1 to 4 show AIS, HAD, SCO-HAD, and SCT-HAD respectively. Ten µg of proteins were loaded in each well. Lanes 5 to 7 show WEY, WEY90H, and WEY95H respectively. Thirty µg of proteins were loaded in each well. The arrow indicates the dioscorin position.

water-extracted yam tuber added to instant foods lowered blood pressure of SHR, and the same for hypertensive subjects. Hence the simple food processing procedure of the prepared PYP and LYP were used to investigate antihypertensive activity on SHRs.

A final concentration of 80% ethanol was used to blend peeled yam tuber and the extracted small molecules, such as simple sugars, organic acids, and pigments, were discarded. The residues saved (alcohol insoluble solids, AIS) contained mainly polysaccharides (possibly including cellulose, hemicellulose, lignin, starch, and pectic substances) and proteins.[21] Figure 2 shows the effects of different amounts of AIS (40 and 60 mg of AIS/kg of SHR) on lowering SHR's blood pressure that followed a single oral administration during 24 h. SBPs in the control were 195.1, 192.3, 198.1, 195.7, and 199.8 mmHg respectively for 2, 4, 6, 8, and 24 h (Fig. 2A). DBPs in the control were 161.2, 157.3, 164.5, 158.8, and 159.7 mmHg respectively for 2, 4, 6, 8, and 24 h (Fig. 2B). The results indicate that AIS at doses of 40 and 60 mg AIS/kg SHR effectively lowered SHR's SBP and DBP and showed significant differences ($p < 0.01$), and higher doses proved even more effective. The negative values showed reduction effects in BP after sample treatment from the calculation equation of $BP_{treated\ sample}$–$BP_{control}$ at the same treatment time. For AIS at 60 mg/kg of SHR oral administration, the highest reductions, of 32.4 mmHg and 31.9 mmHg, for ΔSBP mmHg and ΔDBP mmHg respectively, were reached after 6 or 8 h. The average weight of SHRs was close to 250 g, and the real amount of oral administration was about 15 mg AIS at a dose of 60 mg/kg of SHR. SBP reduction lasted more than 24 h whether 40 or 60 mg of AIS/kg of SHR was tested.

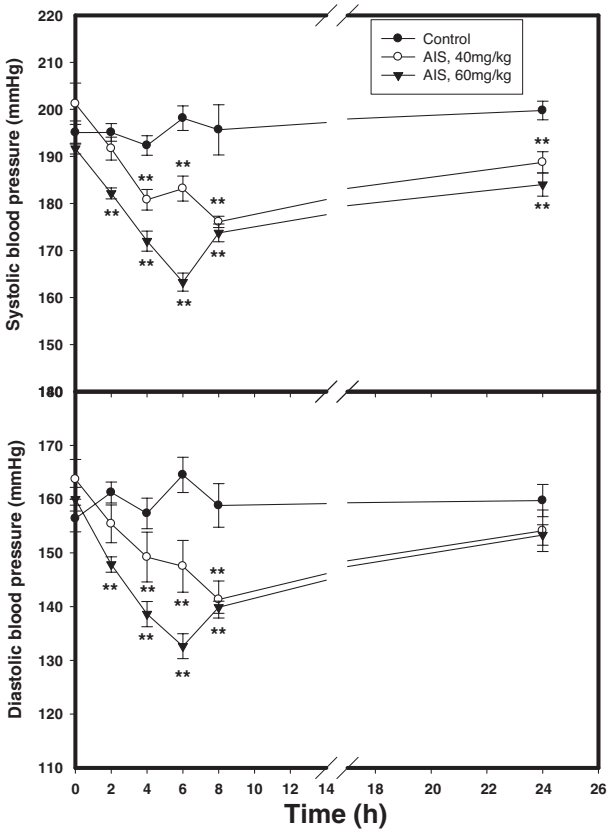

Fig. 2. Effects of Alcohol-Insoluble Solids (AIS) of Powdered-Yam-Products at Doses of 40 mg/kg and 60 mg/kg on Changes Systolic Blood Pressure (A) and Diastolic Blood Pressure (B) of Spontaneously Hypertensive Rats over 24 h after Single Oral Administration.

A difference was considered statistically significant between control and each treatment at the same time after single oral administration when $p < 0.01$ (**).

For storage of an abundant harvest of yam tuber, the HAD procedure might be a suitable method to solve the problem of storage space,[27] and the HAD flour of yam tuber might be used to produce different types of foods. In view of food safety, the cooking procedure is necessary in processed food products. Therefore, the HAD procedure was then performed on fresh, peeled yam tuber slices and on yam slices steam-cooked once (SCO) or steam-cooked twice (SCT) (Fig. 3). The SCO-HAD and SCT-HAD products might be marketable as an instant food. All the powdered products (HAD, 40 mg or 60 mg/kg of SHR; SCO-HAD and SCT-HAD, 80 mg/kg of SHR) were used to investigate antihypertensive activity in SHRs. Figure 4 shows the effects of HAD on changes in SHR's SBP after a single oral administration during 24 h. SBPs in the control were 195.1, 192.3, 198.1, 195.7, and 199.8 mmHg respectively for 2, 4, 6, 8, and 24 h. The effects of HAD on changes in SHR's SBP at a dose of 40 mg/kg of SHR were 199.8, 184.7, 181.1, 187.4, and 193.6 mmHg respectively for 2, 4, 6, 8, and 24 h; dose of 60 mg/kg of SHR, they were 181.1, 175.7, 169.2, 186.4, and 195.7 mmHg respectively for 2, 4, 6, 8, and 24 h (Fig. 4). It was found that HAD at doses of 40 and 60 mg/kg of SHR effectively lowered SHR's SBP and showed significant differences ($p < 0.05$ and $p < 0.01$), and higher doses proved even more effective. In Table 2, the negative values show the reduction effects of BP after sample treatment based on

Fig. 3. Photographs of HAD (A), SCO-HAD (B), and SCT-HAD (C) of Yam Tuber Slices and Powders.

formula $BP_{treated\ sample}-BP_{control}$ at the same treatment time. For HAD at 60 mg/kg of SHR oral administration (Table 2), the highest SBP reduction was at the 6th h (28.9 ± 0.9 mmHg). For SCO-HAD and SCT-HAD, a higher dose, of 80 mg/kg of SHR, was applied (Table 2) in the same experiment. SCO-HAD and SCT-HAD were found to lower SBP of SHR effectively, and the highest SBP reduction was either at the 4th h (SCO-HAD, 19.6 ± 2.6 mmHg) or the 6th h (SCO-HAD, 19.7 ± 2.6 mmHg; SCT-HAD, 16.7 ± 2.7 mmHg). The average weight of SHR was close to 250 g, and the real amount of oral administration was about 20 mg SCO-HAD or SCT-HAD at dose of 80 mg/kg of SHR. It was noted that repeated cooking lowered antihypertensive activity toward SHRs. The order of antihypertensive activity of PYP on SHR was as followed: AIS > HAD > SCO-HAD > SCT-HAD.

Several reports have addressed the biological activities, especially the antioxidant activities, of processed yam products, including the effects of different drying methods (freeze-drying, hot air-drying, and drum-drying) on the antioxidant activity of yam flours,[27] the antioxidant effects of freeze-dried yam powders on hyperhomocysteinemic rats,[28] the antioxidant and hypolipidemic effects of yam-boxthorn noodle in an animal model,[29] the effects of domestic processing (steaming, microwave cooking, baking, frying) on yam steroidal saponins,[30] and the effects of heating yam slices at a 10-fold volume (W/V) of 50–100 °C water for 10 min and then measuring the antioxidant activities in yam crude extracts.[31] This paper is the first to report that different yam products, the PYP of AIS, SCO, or SCT followed by HAD of yam tuber slices, exhibited antihypertensive activity toward SHRs. It has been proposed that dioscorin,[12] mucilage,[32] and natural components in PYP might contribute to the lowering effects on blood pressure of SHR.

The LYP included WEY, WEY90H, or WEY95H (the WEY was heated at 90 or 95 °C for 10 min), and the heated WEY was then stored at 4 °C for 1, 3, and 6 d (WEY90/95H-S1, S3, and S6). The heated WEY might be marketable as a drink. The calculated protein contents in LYP were 154.4 mg of protein/ml using dioscorin as standard. Table 3 shows the antihypertensive activities of LYP toward SHR after a single oral administration. Without heating, the WEY showed good antihypertensive effects on SBP of SHR, and it lasted more than 24 h. The highest SBP reduction was around 25–30 mmHg, and this was maintained from 4–24 h after a single oral administration. The WEY90H and WEY90H-S1 also showed antihypertensive activities

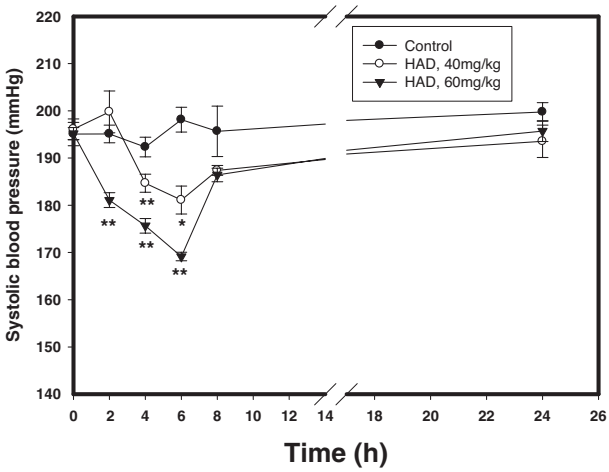

Fig. 4. Effects of Hot-Air Drying (HAD) of Powdered-Yam-Products at Doses of 40 mg/kg and 60 mg/kg on Changes Systolic Blood Pressure of Spontaneously Hypertensive Rats over 24 h after Single Oral Administration.

A difference was considered statistically significant between control and each treatment at the same time after single oral administration when $p < 0.05$ (*) or $p < 0.01$ (**).

toward SHRs which were not significantly different from WEY at the 2nd h or 4th h. However, WEY90H-S3 and WEY90H-S6 at the 4th h, but not at the 2nd h, was significantly different from WEY and had lower antihypertensive activities toward SHR at the same time ($p < 0.001$). WEY95H showed significantly different ($p < 0.05$) and had reduced antihypertensive activities toward SHR in the comparison with WEY at the 4th h, but not at the 2nd h. WEY95H-S1, WEY95H-S3, and WEY95H-S6 showed significantly different ($p < 0.001$) and much lower antihypertensive activities toward SHR in comparison with WEY at the 4th h, but not at the 2nd h. Liu *et al.*[14] reported that purified dioscorin after being heated at 100 °C for 5 min also showed DPPH and hydroxyl radical scavenging activities. It appeared that the heating process at 95 °C, but not at 90 °C, for 10 min might have partially affected the antihypertensive activity of the yam water extracts as compared to the unheated ones. Storage at 4 °C after heating might cause interactions between dioscorin and components in the extracts, such as pigments, carbohydrates, and other natural products, and resulted in protein precipitation, which might have affected the antihypertensive activity of the yam water extracts.

Oral administration of WEY90H on SHR once a day for 30 d was used to investigate antihypertensive effects (Fig. 5). The average SBP reduction in WEY90H was about 10–20 mmHg. There were 21.75, 22.72, 22.42,

Table 2. Effects of Powdered Hot-Air-Dryed (HAD) Yam Tuber Slices and Yam Tuber Slices Steam-Cooked Once (SCO) or Twice (SCT) Followed by HAD on Changes in Systolic Blood Pressure (SBP) of Spontaneously Hypertensive Rats (SHR) by a Single Oral Administration

Hour	HAD[a] (ΔSBP mmHg)[b]		SCO-HAD[a] (ΔSBP mmHg)[b]	SCT-HAD[a] (ΔSBP mmHg)[b]
	40 mg/Kg SHR	60 mg/Kg SHR	80 mg/Kg SHR	80 mg/Kg SHR
2	0	−14.0 ± 1.6	−15.6 ± 5.0	−9.5 ± 4.0
4	−7.7 ± 1.9	−16.6 ± 1.5	−19.6 ± 2.6	−12.0 ± 2.3
6	−17.0 ± 3.0	−28.9 ± 0.9	−19.7 ± 2.6	−16.7 ± 2.7
8	−8.3 ± 1.0	−9.2 ± 1.4	−18.2 ± 3.1	−9.6 ± 5.0

[a]HAD was hot-air dried at 50 °C for 2 d; SCO-HAD was steam-cooked for 40 min once and then hot-air dried at 50 °C for 2 d; SCT-HAD was steam-cooked for 40 min twice and then hot-air dried at 50 °C for 2 d.
[b]The lowering effects on blood pressure changes (ΔBP) were calculated as $BP_{treated\ sample}-BP_{control}$ at the same treatment time after oral administration.

Table 3. Effects of Water Extracts of Yam Tuber (WEY) Heated at 90 °C for 10 min (WEY90H) or 95 °C for 10 min (WEY95H) and Then Stored at 4 °C for 1 d (WEY90H-S1, WEY95H-S1), 3 d, and 6 d on Changes in Systolic Blood Pressure (SBP) of Spontaneously Hypertensive Rats (SHR) by a Single Oral Administration

Hour	WEY[a] (ΔSBP mmHg)[b]	WEY90H[a] (ΔSBP mmHg)	WEY90H-S1[a] (ΔSBP mmHg)	WEY90H-S3[a] (ΔSBP mmHg)	WEY90H-S6[a] (ΔSBP mmHg)
	1 ml/Kg SHR[c]	1 ml/Kg SHR	1 ml/Kg SHR	1 ml/Kg SHR	1 ml/Kg SHR
2	−16.3 ± 1.3	−15.8 ± 3.3	−15.8 ± 3.1	−11.2 ± 4.7	−13.2 ± 2.0
4	−30.3 ± 1.0	−29.2 ± 2.0	−29.2 ± 2.0	−21.0 ± 4.3***	−18.9 ± 1.1***
6	−29.5 ± 3.2	—[d]	—[d]	—[d]	—[d]
8	−26.3 ± 2.2	—[d]	—[d]	—[d]	—[d]
24	−25.2 ± 2.1	—[d]	—[d]	—[d]	—[d]

Hour	WEY[a] (ΔSBP mmHg)[b]	WEY95H[a] (ΔSBP mmHg)	WEY95H-S1[a] (ΔSBP mmHg)	WEY95H-S3[a] (ΔSBP mmHg)	WEY95H-S6[a] (ΔSBP mmHg)
	1 ml/Kg SHR[c]	1 ml/Kg SHR	1 ml/Kg SHR	1 ml/Kg SHR	1 ml/Kg SHR
2	−16.3 ± 1.3	−15.9 ± 3.4	−16.8 ± 2.8	−14.5 ± 3.8	−16.5 ± 3.1
4	−30.3 ± 1.0	−25.7 ± 2.4*	−24.2 ± 2.2***	−21.1 ± 2.5***	−16.8 ± 3.8***
6	−29.5 ± 3.2	—[d]	—[d]	—[d]	—[d]
8	−26.3 ± 2.2	—[d]	—[d]	—[d]	—[d]
24	−25.2 ± 2.1	—[d]	—[d]	—[d]	—[d]

[a]The WEY was water extracts of yam tuber without heat treatment. WEY90H and WEY95H, the WEY were heated at 90 °C or 95 °C for 10 min. WEY90H/95H-S1, the WEY were heated at 90 °C or 95 °C for 10 min and then stored at 4 °C for 1 d. WEY90H/95H-S3, the WEY were heated at 90 °C or 95 °C for 10 min and then stored at 4 °C for 3 d. WEY90H/95H-S6, the WEY were heated at 90 °C or 95 °C for 10 min and then stored at 4 °C for 6 d.
[b]The lowering effects on blood pressure changes (ΔBP) were calculated as $BP_{treated\ sample}-BP_{control}$ at the same treatment time after oral administration. A difference was considered statistically significant between WEY and WEYH and between WEY and WEYH-S when $p < 0.05$ (*), or $p < 0.01$ (**), or $p < 0.001$ (***).
[c]The 1 ml water extracts were calculated to contain 154.4 mg of proteins (using dioscorin as standard).
[d]no measurement.

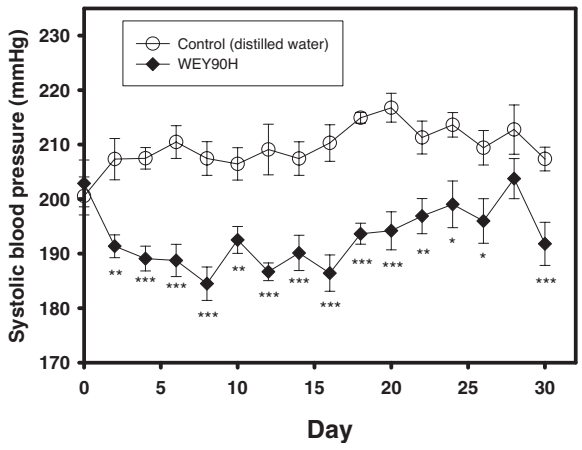

Fig. 5. Effects of Water Extracts of Yam Tuber Heated at 90 °C for 10 min (WEY90H) on Systolic Blood Pressure of Spontaneously Hypertensive Rats by Oral Administration (1 ml/kg of SHR) Once a Day for 30 d.

The 1-ml extracts were calculated to contain 154.4 mg proteins using dioscorin as standard and 1-ml of distilled water was used in a control experiment. A difference was considered statistically significant between control and treated group when $p < 0.05$ (*), or $p < 0.01$ (**), or $p < 0.001$ (***).

23.87, 21.27, 22.57, and 15.57 mmHg SBP reduction respectively for the 6th, 8th, 12th, 16th, 18th, 20th, and 30th d. Compared to the control (distilled water administration), the WEY90H showed significantly different (the 24th and 26th d, $p < 0.05$; 2nd, 10th, and 22th d, $p < 0.01$; 4th, 6th, 8th, 12th, 14th, 16th, 18th, 20th, and 30th d, $p < 0.001$) and clearly antihypertensive activity on SHR during one month. This is the first report that heated LYP can lower SHR blood pressure.

In conclusion, powdered yam products and the liquid yam products were found to have antihypertensive activities in SHRs. This might be the useful information for food processing in the development of functional foods for blood pressure regulation.

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