Effects of tuber storage protein of yam (*Dioscorea alata* cv. Tainong No. 1) and its peptic hydrolyzates on spontaneously hypertensive rats

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Abstract: Yam storage protein (YSP) was purified from tubers of *Dioscorea alata* L. Tainong No. 1 (TN1) to homogeneity by DE-52 ion-exchange chromatography. The short-term (24 h) and long-term (25 days) antihypertensive effects of YSP-TN1 and its peptic hydrolyzates (PH-TN1) were measured in spontaneously hypertensive rats (SHRs). For 24-h antihypertensive measurements, SHRs (age 10 weeks, body weight from 240 to 250 g) were administered orally once (YSP-TN1 and PH-TN1, 40 mg kg^{-1} SHR) to measure the mean blood pressure (MBP), systolic blood pressure (SBP) and diastolic blood pressure (DBP). For a long-term antihypertensive measurement, SHRs (age 12 weeks, body weight from 250 to 270 g) were administered orally once a day for 25 days (YSP-TN1, 40 mg kg^{-1} SHR) to measure SBP, DBP and MBP. Captopril (10 or 15 mg kg^{-1} SHR) was used as a positive control. It was found that short-term administration of 40 mg kg^{-1} SHR of YSP-TN1 and PH-TN1 effectively lowered SHRs' MBP, SBP and DBP (For YSP-TN1, the lowest blood pressure was reached in the fourth hour and for PH-TN1 in the eighth hour). The lasting effects of PH-TN1 on reduced SHRs' BP were better than those of YSP-TN1 for one oral administration. For oral administration of 40 mg YSP-TN1 kg^{-1} SHR, the reduced MBP was 21.5 mmHg, which was comparable to 25.2 mmHg (the fourth hour) of 10 mg captopril kg^{-1} SHR oral administration. For oral administration of 40 mg PH-TN1 kg^{-1} SHR, the reduced MBP was 33.7 mmHg, comparable to 38.4 mmHg (the fourth hour) of 15 mg captopril kg^{-1} SHR. For long-term 25-day oral administration of 40 mg YSP-TN1 kg^{-1} SHR once a day, it was found that a feeding trial of YSP-TN1 effectively lowered SHRs' SBP, DBP and MBP. The greatest reduction in SHRs' blood pressure was reached on the ninth day, for the reduced SBP, 27.7 mmHg; for the reduced DBP, 28.3 mmHg; and for the reduced MBP, 27.5 mmHg.

Keywords: antihypertension; diastolic blood pressure (DBP); means of blood pressure (MBP); peptic hydrolyzates; systolic blood pressure (SBP); spontaneously hypertensive rat (SHR); storage protein; yam

INTRODUCTION

Several peptide-derived angiotensin-converting-enzyme (ACE) inhibitors have been used in the animal model of spontaneously hypertensive rats (SHRs) to evaluate the antihypertensive effects.[1–11] Sato *et al*.[7] pointed out that three dipeptides, including AW ($IC_{50} = 18.8\,\mu mol\,L^{-1}$), VW ($IC_{50} = 3.3\,\mu mol\,L^{-1}$) and LW ($IC_{50} = 23.6\,\mu mol\,L^{-1}$), were potential ACE inhibitory peptides. However, none of them were able effectively to reduce the blood pressure of SHRs in animal models. Fujita *et al*.[3] also found that the octapeptides of FFGRCVSP ($IC_{50} = 0.4\,\mu mol\,L^{-1}$) and ERKIKVYL ($IC_{50} = 1.2\,\mu mol\,L^{-1}$) were potent ACE inhibitors, but none of them were effective in animal models to reduce the blood pressure of SHRs. Based on the above results, these potential ACE inhibitory peptides might be hydrolyzed in advance by the rat's gastrointestinal proteases and lose their antihypertensive effects on SHR *in vivo*.

Dried slices of yam tuber are frequently used in Chinese herbal medicine and fresh tuber is also a staple food in West Africa, Southern Asia and the Caribbean. Yam mucilage has been reported to exhibit antioxidant[12] and ACE inhibitory activities.[13] The tubers of yam storage protein (YSP) account for about 90% of the extractable water-soluble proteins from different species (*Dioscorea batatas*, *D. alata*, *D. pseudojaponica*) as estimated by the immunostaining method[14] and all YSP exhibited carbonic anhydrase and trypsin inhibitor activities.[14,15] We also found that

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YSP exhibited both dehydroascorbate reductase and monodehydroascorbate reductase activities and might respond to environmental stresses.[16] The purified YSP from yam (*Dioscorea batatas* Decne) tuber exhibited antioxidant activities against different radicals.[17] Although there has been no report of dried slices of yam tuber being used in Chinese herbal medicine for antihypertensive treatments, a recent study of ours showed that both YSP and its peptic hydrolyzates exhibited ACE inhibitory activities *in vitro*.[18] It was proposed that in the process of cooking Chinese herbal medicines, the YSP in the dried yam tuber was denatured and heat-stable components were only extracted and therefore no available YSP was ingested. In this work, YSP-TN1 and its peptic hydrolyzates (PH-TN1) were used to evaluate short-term (24 h) and long-term (25 days) effects of the antihypertension activities on SHR to observe the changes in systolic blood pressure (SBP), diastolic blood pressure (DBP) and means of blood pressure (MBP).

EXPERIMENTAL

Materials

Captopril was purchased from Calbiochem (San Diego, CA, USA). DE-52 anion-exchange resin was supplied by Whatman (New York, NJ, USA); Other chemicals and reagents were obtained from Sigma Chemical (St Louis, MO, USA).

Purification of yam tuber storage proteins

Fresh yam (*Dioscorea alata* L. Tainong No. 1) tubers were purchased from a local wholesaler. After being washed and peeled, the tubers were cut into strips for storage protein purification as described in previous reports.[14–18] Yam tubers were homogenized with four volumes (w/v) of 50 mmol L^{-1} Tris–HCl buffer (pH 8.3). After centrifugation at 12 500 × *g* for 30 min, the supernatants were saved and loaded directly on to a DE-52 ion-exchange column. After being washed with three column volumes of 50 mmol L^{-1} Tris–HCl buffer (pH 8.3), the adsorbed YSP was eluted batchwise with the same washing buffer containing 150 mmol L^{-1} NaCl. The eluted fraction was collected and concentrated with Ultrafree-4 (molecular weight cutoff 5 kDa) (Millipore, Bedford, MA, USA). The concentrated YSP solution was dialyzed against deionized water overnight and lyophilized for further use.

Short-term effects of YSP-TN1 and PH-TN1 on SHR

The effects of orally administered YSP-TN1 and PH-TN1 by feeding tube (2.0 × 80 mm) on the reduced SBP, the reduced DBP and the reduced MBP were determined according to the method of Chen *et al*.[8] All animal experimental procedures followed the published guidelines.[19] The male SHRs (age 8 weeks) (National Laboratory Animal Center, Taipei, Taiwan) were housed individually in steel cages kept at 24 °C with a 12-h light–dark cycle and had free access to a standard laboratory diet [5001 Rodent Diet, containing ground corn, dehulled soybean meal, dried beet pulp, fish meal, ground oats, brewer's dried yeast, cane molasses, dehydrated alfalfa meal, dried whey, wheat germ, porcine meat meal, wheat middlings, animal fat preserved with butylated hydroxyanisole (BHA), salt, calcium carbonate, choline chloride, cholecalciferol, vitamin A acetate, folic acid, pyridoxine hydrochloride, DL-methionine, thiamine mononitrate, calcium pantothenate, nicotinic acid, DL-α-tocopheryl acetate, cyanocobalamin, riboflavin, ferrous sulfate, manganous oxide, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate) (PMI, St Louis, MO, USA) and water. After being housed for 10 weeks, the SHRs' weight ranged from 240 to 250 g and SBP reached 180 mmHg. They were randomly divided into five groups (five rats per group) of control, captopril, YSP-TN1 (for MBP, DBP and SBP determinations) and PH-TN1 (for MBP, DBP and SBP determinations). The peptic hydrolyzates of yam tuber storage proteins were treated according to the method of Hsu *et al*.[18] The hydrolyzates were passed through a pepstatin–agarose affinity column to adsorb pepsin. The unbound solutions were then lyophilized as PH-TN1 for experiments. For a short-term antihypertensive experiment, 0.5 mL of 20 and 40 mg YSP-TN1 kg^{-1} SHR or 0.5 mL of 40 mg PH-TN1 kg^{-1} SHR were administered orally once and the tail blood pressure was measured four times at each desired time during 24 h using an indirect blood pressure meter (BP-98A, Softron, Tokyo, Japan) for each treatment. Before each blood pressure measurement, SHRs were warmed for 10 min in a 39 °C thermostated box. The MBP was automatically calculated by machine using the formula of 1/3[(SBP − DBP) + DBP]. As a positive control, 10 or 15 mg captopril kg^{-1} SHR was used. The changes in blood pressure (BP, including MBP, SBP and DBP) was calculated as $BP_{\text{treated sample}} - BP_{\text{normal saline}}$.

Long-term effects of YSP-TN1 on SHR

After being housed for 12 weeks, the SHRs' weight ranged from 250 to 270 g and SBP reached 190 mmHg. A 0.5 mL volume of 40 mg YSP-TN1 kg^{-1} was administered orally to SHRs (five rats per group) once a day for 25 days and their blood pressure was measured every day. A 0.5 mL volume of normal saline was used as a negative control.

Statistical analysis

Student's *t*-test was used for comparisons between the control and each treatment at the same time interval. A difference was considered statistically significant when $P < 0.05$ (*) or $P < 0.01$ (**).

RESULTS AND DISCUSSION

YSP has been reported to be a glycoprotein with a molecular mass of 28 kDa.[14–16] Both it

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and its peptic hydrolyzates have been proven to exhibit ACE inhibitory activities *in vitro*.[18] In the literature, potential peptide-derived ACE inhibitors were reported to lower the blood pressure of SHRs effectively, but some cases were not successful.[3,7] Although there are no reports of dried slices of yam tuber being used in Chinese herbal medicine for antihypertensive treatments, a recent report of ours showed that both YSP and its peptic hydrolyzates exhibited ACE inhibitory activities *in vitro*.[18] It was proposed that in the process of cooking Chinese herbal medicines, the YSP in the dried slices of yam tuber was denatured and heat-stable components were extracted and therefore no available YSP was ingested. This is the first report that oral administration of a native protein and its peptic hydrolyzates from yam tubers exhibits antihypertensive activity on SHRs. The purity of YSP-TN1 (>99%) in this research was the same as in previous reports.[15–18]

Short-term effects of YSP-TN1 on SHRs' MBP

YSP-TN1 was used to evaluate the short-term effects on SHRs' MBP. The MBP was calculated automatically using the formula 1/3[(SBP − DBP) + DBP] by blood pressure meter. Millar *et al*.[20] published a seminal paper suggesting that stroke was best predicted by mean blood pressure. Volumes of 0.5 mL of YSP-TN1 (20 and 40 mg kg^{-1} SHR) were prepared for determining antihypertensive activity. A 0.5 mL volume of captopril (10 mg or 15 mg kg^{-1} SHR) was used as a positive control and 0.5 mL of normal saline as a negative control. The reduced MBP was calculated as $MBP_{\text{treated sample}} - MBP_{\text{normal saline}}$. Figure 1 shows the reduced MBP ($MBP_{\text{treated sample}} - MBP_{\text{normal saline}}$) of SHR after one oral administration of YSP-TN1. The results in Fig. 1 revealed that 20 or 40 mg YSP-TN1 kg^{-1} SHR could effectively lower the SHRs' MBP and the lowest MBP was reached in the fourth hour. Administration of 20 and 40 mg YSP-TN1 kg^{-1} SHR could lower the MBP by 12.4 and 21.5 mmHg, respectively, in comparison with 25.2 mmHg with 10 mg captopril kg^{-1} SHR.

Short-term effects of YSP-TN1 on SHRs' SBP and DBP

Figure 2 shows the effects of 40 mg YSP-TN1 kg^{-1} SHR on SHRs' SBP (A) and DBP (B) after one oral administration during 24 h. Captopril (15 mg kg^{-1} SHR) was used as a positive control and normal saline was used as a negative control. A difference was considered statistically significant between normal saline and treated groups when $P < 0.05$ (*) or $P < 0.01$ (**). The results in Fig. 2(A) clearly revealed that the YSP-TN1-treated group had a lower SBP than the negative control group at 2 and 4 h after an oral administration and showed a significant difference (2 h, $P < 0.05$; 4 h, $P < 0.01$). The results in Fig. 2(B) clearly revealed that the YSP-TN1-treated group also had a lower DBP than the negative control group at 2 and 4 h after an oral administration and showed

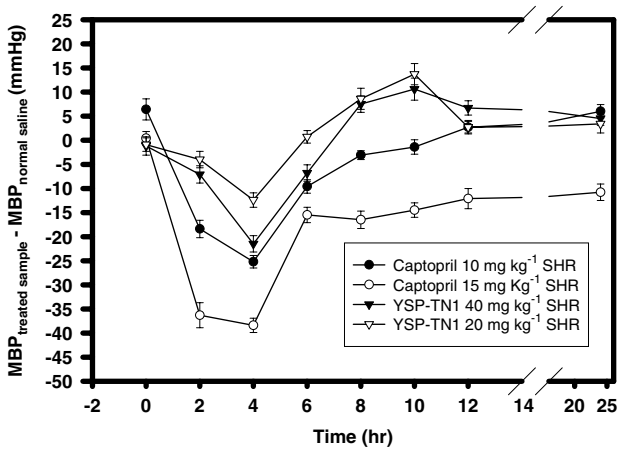

Figure 1. The short-term effects (24 h) of YSP-TN1 on the changes in means of blood pressure of SHR after one oral administration. Volumes of 0.5 mL of YSP-TN1 (20 and 40 mg kg^{-1} SHR) were prepared for determining antihypertensive activity. Captopril (10 and 15 mg kg^{-1} SHR) was used as a positive control and normal saline as a negative control. The reduced MBP was calculated as ($MBP_{\text{treated sample}} - MBP_{\text{normal saline}}$) and was expressed as ΔMBP.

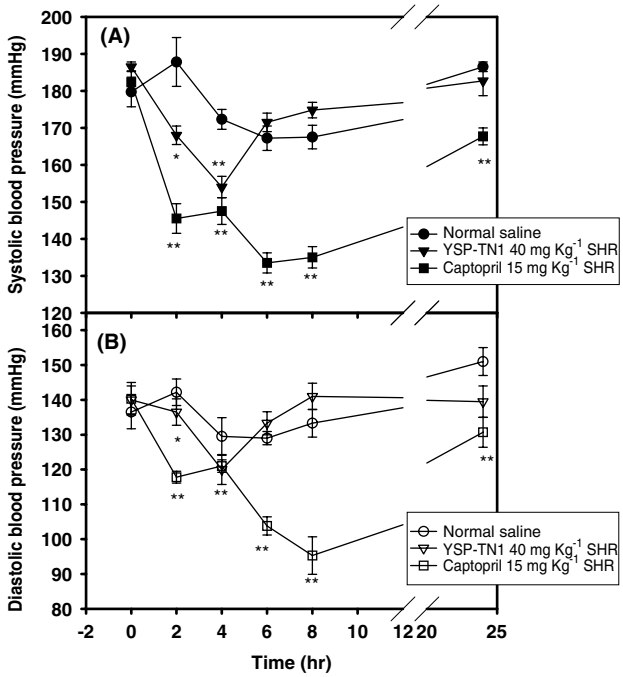

Figure 2. The short-term effects (24 h) of YSP-TN1 (40 mg kg^{-1} SHR, A) and captopril (15 mg kg^{-1} SHR, B) on the systolic blood pressure (A) and diastolic blood pressure (B) of SHR after one oral administration. Captopril (15 mg kg^{-1} SHR) was used as a positive control and normal saline as a negative control. A difference was considered statistically significant between control and treated groups when $P < 0.05$ (*) or $P < 0.01$ (**).

a significant difference (2 h, $P < 0.05$). The results in Figs 1 and 2 show that YSP-TN1 had short-term antihypertensive activity on SHR. It may be that the YSP-TN1 was quickly hydrolyzed into potent ACE inhibitory peptides in the rat's gastrointestinal digestion, which might be absorbed and circulated to lower the SHRs' blood pressure (especially the SBP) in the first 2–4 h, after which the original hypertensive blood pressure would return.

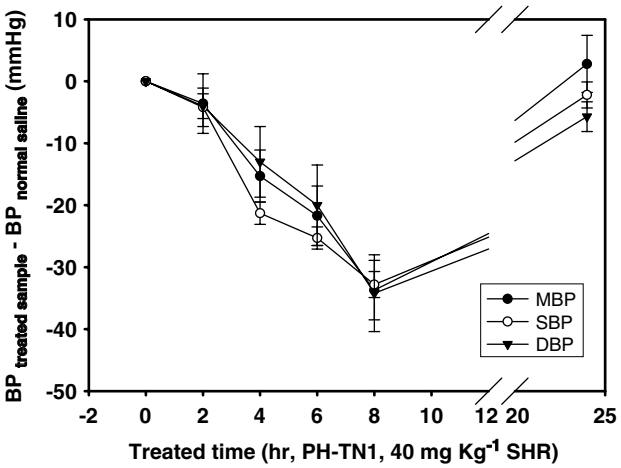

Figure 3. The short-term effects (24 h) of peptic hydrolyzates of yam tuber storage proteins (PH-TN1, 40 mg kg^{-1} SHR) on the changes in MBP, SBP and DBP of SHR after one oral administration. Normal saline was used as a negative control. The reduced BP was calculated as ($BP_{treated\ sample} - BP_{normal\ saline}$).

Table 1. Short-term effects of peptic hydrolyzates of yam storage protein (40 mg kg^{-1} SHR) on the changes in MBP, SBP and DBP after oral administrations during 24 h

Time (h)[a]	ΔMBP[b] (mmHg)	ΔSBP (mmHg)	ΔDBP (mmHg)
2	−3.6	−4.2	−4
4	−15.3	−21.3	−13
6	−21.7	−25.3	−20
8	−33.7	−32.8	−34.2
24	2.8	−2.2	−5.7

[a] Oral administration of peptic hydrolyzates of yam storage protein (40 mg kg^{-1} SHR).
[b] The reduced MBP was calculated as ($MBP_{treated\ sample} - MBP_{normal\ saline}$) and was expressed as ΔMBP.

Short-term effects of PH-TN1 on SHRs' MBP, SBP and DBP

It was interesting to investigate whether the YSP, predigested by a gastrointestinal enzyme such as pepsin, also exhibited antihypertensive effects *in vivo*. Figure 3 and Table 1 show the short-term effects (24 h) of the peptic hydrolyzates of yam tuber storage proteins (PH-TN1, 40 mg kg^{-1} SHR) on the reduced MBP, the reduced SBP and the reduced DBP of SHR after one oral administration during 24 h. The reduced BP was calculated as $BP_{treated\ sample} - BP_{normal\ saline}$. The results in Fig. 3 reveal that the PH-TN1-treated SHR showed greatly reduced MBP, SBP and DBP and the lowest one in the MBP, SBP and DBP was reached in the eighth hour. Table 1 shows the changes in MBP, SBP and DBP after one oral administration of PH-TN1 after 2, 4, 6, 8 and 24 h. The reduced MBP was −3.6, −15.3, −21.7 and −33.7 mmHg for 2, 4, 6 and 8 h, respectively, before returning to the original blood pressure after 24 h; The reduced MBP was −3.6, −15.3, −21.7 and −33.7 mmHg for 2, 4, 6, 8 h, respectively, and back to the original blood pressure after 24 h; the reduced DBP was −4.0, −13.0, −20 and −34.2 mmHg for 2, 4, 6, 8 h, respectively, and back to the original blood pressure after 24 h. In our previous study, smaller molecular sizes of peptides were produced in peptic TN1 hydrolyzates and the PH-TN1 exhibited higher ACE inhibitory activities than unhydrolyzed YSP-TN1 at the same weight.[18] The lasting effects of PH-TN1 on reduced SHRs' BP were better than those of YSP-TN1 for one oral administration. This meant that the pre-hydrolysis of YSP by pepsin might be beneficial for antihypertension. For oral administration of 40 mg PH-TN1 kg^{-1} SHR, the reduced MBP was 33.7 mmHg (the eighth hour), which was comparable to that of 38.4 mmHg (Fig. 1, the fourth hour) of 15 mg captopril kg^{-1} SHR.

Long-term effects of YSP-TN1 on SHR

Most papers[3,6–10] reported short-term antihypertensive activity on SHR after one oral administration. The YSP-TN1 exhibiting short-term antihypertensive activity by lowering MBP, SBP and DBP on SHR was used in a long-term trial on SHR. Figure 4(A) shows the effects of 40 mg YSP-TN1 kg^{-1} SHR on SHRs' SBP by oral administration once a day for 25 days. From the results in Fig. 4(A), the YSP-TN1-treated group showed a clearly lower and significantly different SBP [Fig. 4(A), $^{*}P < 0.05$ and $^{**}P < 0.01$] from the negative control group (normal saline) during 25-day treatments. The greatest reduction in SHRs' SBP was reached on the ninth day with 27.7 mmHg [Fig. 4(A)]. Figure 4(B) shows the effects of YSP-TN1 40 mg kg^{-1} SHR on SHRs' DBP by oral administration once a day for 25 days. It was found that the YSP-TN1-treated group had similar effects on lowering DBP during 25-day treatments [Fig 4(B)]. The eighth and ninth days showed significantly different DBPs [Fig. 4(B), $^{*}P < 0.05$] from those of the negative control group (normal saline). The greatest DBP reduction was reached on the ninth day with 28.3 mmHg [Fig 4(B)]. Figure 4(C) shows the effects of YSP-TN1 40 mg kg^{-1} SHR on SHRs' MBP by oral administration once a day for 25 days. It is clear that the YSP-TN1-treated group had lower MBP during the 25-day treatments [Fig 4(C)]. The eighth, ninth, 16th and 17th days showed significantly different MBP [Fig 4(C), $^{*}P < 0.05$] from the control group. The above results make it clear that YSP-TN1 also showed long-term antihypertensive effects on SHR.

CONCLUSIONS

The results of the present study show that oral administration of YSP-TN1 and its peptic hydrolyzates (PH-TN1) exhibited short-term (Figs 1–3, Table 1) and long-term (Fig. 4) antihypertensive activity in SHRs. This is the first report that the direct administration of native protein, the yam tuber storage protein (YSP-TN1), exhibits antihypertensive activity *in vivo*. Although no report on the antihypertensive effects of dried slices of yam tuber used in Chinese herbal medicine has appeared, the recent report of ours

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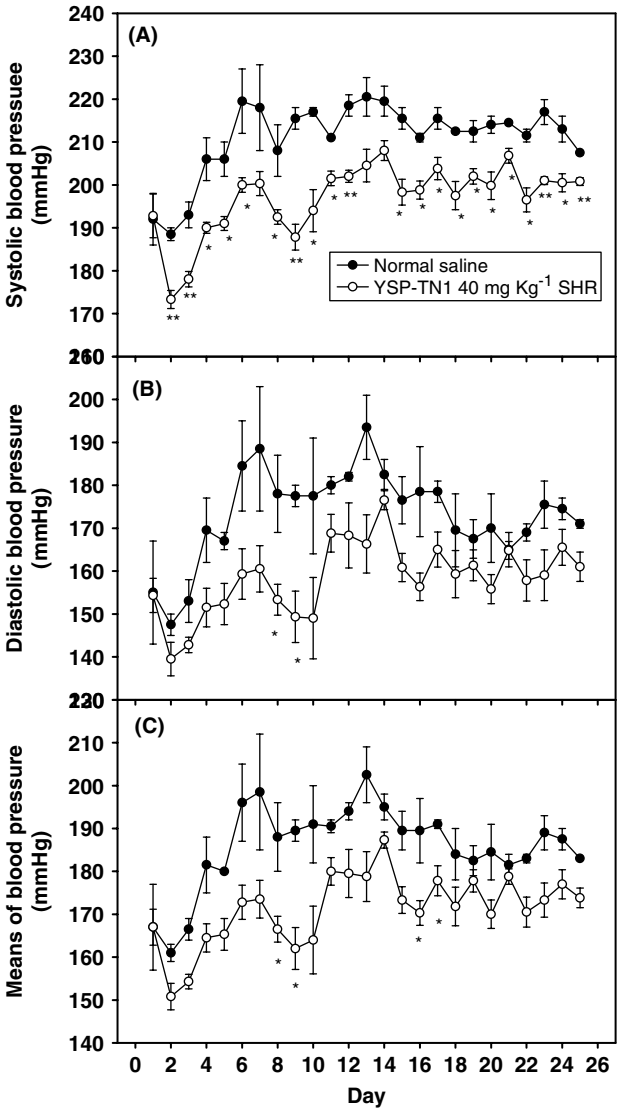

Figure 4. The effects of YSP-TN1 (40 mg kg^{-1} SHR) on (A) systolic blood pressure, (B) diastolic blood pressure and (C) means of blood pressure of SHR by oral administration once a day for 25 days. Normal saline was used as a negative control. A difference was considered statistically significant between control and treated groups when $P < 0.05$ (*) or $P < 0.01$ (**).

showed that both YSP and its peptic hydrolyzates exhibited ACE inhibitory activities *in vitro*. It was proposed that in the process of cooking Chinese herbal medicines, the YSP in the dried slices of yam tuber was denatured and heat-stable components were extracted and therefore none of the available YSP was ingested. The YSP, with its antihypertensive activity in SHRs, might be developed into as a health food, in forms such as yam cookies and yam flour, for regulating blood pressure.

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