Research Article

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Feeding trial of instant food containing lyophilised yam powder in hypertensive subjects

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Abstract

BACKGROUND: It was reported in a previous paper that the yam tuber storage protein dioscorin exhibited antihypertensive effects on spontaneously hypertensive rats. The aim of the present study was to evaluate and compare the effects of packets of instant food (30 g) with (treated meal) and without (placebo) lyophilised yam powder on hypertensive subjects.

RESULTS: A placebo-controlled feeding trial was conducted daily for 5 weeks (stage 1), followed by a 1 week washout and then a 5 week crossover (stage 2). Twenty-one subjects finished the trial. One packet of treated meal contained 140 ± 2.54 mg of dioscorin according to enzyme-linked immunosorbent assay. The blood pressure results of the treated meal and placebo groups at stage 1 end *versus* originals, but not at stage 2 end *versus* stage 2 beginning, were significantly different by the paired *t* test. Systolic (SBP) and diastolic (DBP) blood pressure readings after treated meal intervention, but not after placebo intervention, differed significantly from the original values based on one-way analysis of variance followed by the *post hoc* Tukey test; the reductions in SBP and DBP were 6.52 and 4.76 mmHg respectively. The feeding trial did not appear to affect serum lipid profiles or other biochemical measurements of cardiovascular risk.

CONCLUSION: Intake of an instant food containing 140 mg of dioscorin over 5 weeks had a regulating effect on human blood pressure.

Keywords: antihypertensive effect; blood pressure; crossover; dioscorin; feeding trial; yam

INTRODUCTION

A number of risk factors are associated with stroke, including age, gender, elevated cholesterol, smoking, alcohol, excess weight, race, family history and hypertension.[1] Although some of these risk factors cannot be modified, one factor that can be controlled and has the greatest impact on the aetiology of stroke is high blood pressure.[2] Several classes of pharmacological agents have been used in the treatment of hypertension. One class of antihypertensive drugs known as angiotensin I-converting enzyme (ACE) inhibitors (i.e. peptidase inhibitors) is associated with a low rate of adverse side effects and is the preferred class of antihypertensive agents for treating patients with concurrent secondary diseases.[3]

Several peptide-derived ACE inhibitors have been used in the animal model of spontaneously hypertensive rats (SHRs) to evaluate their antihypertensive effects.[4–11] We have reported previously that the yam tuber storage protein dioscorin exhibits ACE-inhibitory[12] and antihypertensive[13] effects on SHRs, antioxidant activity[14,15] and immunomodulatory activities.[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, southern Asia and the Caribbean. Chen and Lin[17] reported that the content of crude protein (wet weight basis) in different yam species ranged from 36 to 72.8 g kg^{-1}, while Chou *et al*.[18] reported that Tainong No. 1 yam contained 25.8 g kg^{-1} crude protein.

Packets of instant food with (treated meal) and without (placebo) lyophilised yam powder were made specifically for the present study, the goals of which were firstly to evaluate

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the antihypertensive effects on SHRs and secondly to conduct a feeding trial in hypertensive subjects.

EXPERIMENTAL

Materials

The instant foods with (treated meal) and without (placebo) lyophilised yam powder were provided by AGV Products Corp. (Chiayi, Taiwan). One packet of instant food (30 g) was mixed with 150–200 mL of warm water as a morning drink in the feeding trial. To study the antihypertensive effects on SHRs, each instant food portion was mixed with small amounts of water to make a dough-like paste, dried at 37 °C and then divided into pieces for oral administration. The treated meal and placebo had a similar appearance and flavour and were packaged in aluminium foil. Total calorie contents were calculated as 127.6 and 126.4 kcal per packet for the treated meal and placebo respectively. Proximate compositions per packet were as follows: for the treated meal, crude protein, crude lipid and crude carbohydrate contents of 1.7, 2.8 and 23.9 g respectively; for the placebo, crude protein, crude lipid and crude carbohydrate contents of 1.0, 2.9 and 24.1 g respectively. Chemicals and reagents for enzyme-linked immunosorbent assay (ELISA) were from Sigma Chemical Co. (St Louis, MO, USA).

Use of ELISA to determine dioscorin in treated meal

Each packet (30 g) of treated meal or placebo was extracted overnight with 600 mL of phosphate-buffered saline (PBS) and gentle shaking. After centrifugation at 10 000 × *g* for 10 min, supernatants were diluted with PBS in the range 500–2000-fold for dioscorin quantification. Dioscorin from yam tuber was purified according to previous reports.[12–16] The purified dioscorin was used in the assay to plot a standard curve in order to quantify dioscorin in the treated meal. The polyclonal antibody against yam tuber dioscorin was raised from mouse serum. The ELISA procedure[19] was modified as follows. A 100 µL aliquot of extracted solution, standard curve solution (purified dioscorin was prepared at 0.153, 0.313, 0.625 and 1.250 µg mL^{-1}) or PBS was loaded into a high-binding 96-well microtitre plate (Nunc MaxiSorp Type F, Roskilde, Denmark), covered with an adhesive strip and incubated at 37 °C for 2 h, then washed three times (10 min each) with 200 µL of PBST (PBS containing 0.5 g L^{-1} Tween 20) and blocked with 100 µL of 2.5 g L^{-1} gelatin in NET (NaCl, ethylene diamine tetraacetic acid, Tris, Tween 20) solution at 37 °C for 30 min. A 100 µL aliquot of anti-dioscorin polyclonal antibody solution (5000-fold dilution with 2.5 g L^{-1} gelatin in NET solution) was added at 37 °C for 1 h and the plate was washed three times (10 min each) with 200 µL of PBST. Thereafter a 100 µL aliquot of anti-mouse IgG conjugated with horseradish peroxidase (Vector Labs Inc., CA, USA) solution (2500-fold dilution with 2.5 g L^{-1} gelatin in NET solution) was added at 37 °C for 1 h and the plate was washed again using 200 µL of PBST. A 100 µL aliquot of staining solution (1 mg of 3,3′,5,5′-tetramethylbenzidine was dissolved in 1 mL of dimethylsulfoxide, 5 µL of 300 g L^{-1} hydrogen peroxide was added and the solution was made up to 10 mL with 50 mmol L^{-1}PB, pH 5) was added in darkness and allowed to react at 37 °C for 30 min. The reaction was then stopped with 25 µL of 1 mol L^{-1} HCl and the absorbance at 450 nm was measured. Means of triplicates (mean ± standard deviation) were measured.

Effects of antihypertensive activity of treated meal and placebo on SHRs

Before the feeding trial, effects of the treated meal and placebo on the systolic blood pressure (SBP) and diastolic blood pressure (DBP) of SHRs were determined using the method of Lin *et al*.[13] All animal experimental procedures followed the published guidelines.[20] Twenty-four 8-week-old male SHRs (National Laboratory Animal Center, Taipei, Taiwan) were housed individually in steel cages kept at 24 °C with a 12/12 h light/dark cycle and had free access to a standard laboratory diet (5001 Rodent Diet, PMI, St Louis, MO, USA) and water. After being housed for 10 weeks, they were randomly divided into three groups of eight rats each, i.e. a control group, a treated meal group and a placebo group. Each instant food portion was mixed with small amounts of water to make a dough-like paste, dried at 37 °C and then divided into pieces for oral administration daily for 4 weeks at a concentration of 2.5 g kg^{-1} SHR (suspended in 0.5 mL of water). Only 0.5 mL of water was administered orally to the control group. The tail blood pressure of SHRs was measured twice a week. An indirect blood pressure meter (BP-98A, Softron Co. Ltd, Tokyo, Japan) was used to measure SBP and DBP four times at each determination for each treatment.

Subjects and feeding trial

The subject recruitment and feeding trial design were approved by the IRB of Taipei Medical University (approval number P950049). Figure 1 shows a summary of the experimental design. Volunteers were 20–50-year-old males and females with pre-hypertension or hypertension, including SBP between 130 and 140 mmHg or higher than 140 mmHg or DBP between 85 and 90 mmHg or higher than 90 mmHg, as confirmed by a physician in internal medicine at Taipei Medical University Hospital. Key exclusion criteria included pregnant and lactating women, people who had received trace element supplements in the previous 3 months, people undergoing gastric or diuretic treatments and patients with acute renal failure or who had recently had surgery or acute infections. Subjects were informed of the purposes of the study, were free to ask questions throughout the study and signed an informed consent form witnessed by one of the investigators. The study was designed as a randomised, placebo-controlled crossover feeding trial. Subjects ($n = 27$) were randomly assigned into two groups (Fig. 1). The treated meal or placebo was intervened as a morning drink daily for 5 weeks as stage 1, followed by a washout stage for 1 week, and the routine was then crossed over for another 5 weeks as stage 2. Blood pressure was measured using a mercurial blood pressure analyser twice a week at a fixed place and time. Before blood pressure measurement, each subject was asked to rest for at least 15 min. Blood pressure readings were taken twice and averaged. The original blood pressure was measured after each subject was assigned a feeding group. Eating habits were not controlled during intervention. Venous blood collection was performed three times for each subject (Fig. 1) at the beginning of assignment and at the end of the 5 week intervention. Each venous blood sample was collected and assayed for serum lipid profiles and other biochemical measurements of cardiovascular risk such as total cholesterol (TC), triglyceride (TG), glutamyl oxaloacetic transaminase (GOT), glutamyl pyruvic transaminase (GPT), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) by the Department of Laboratory Medicine of Taipei Medical University Hospital.

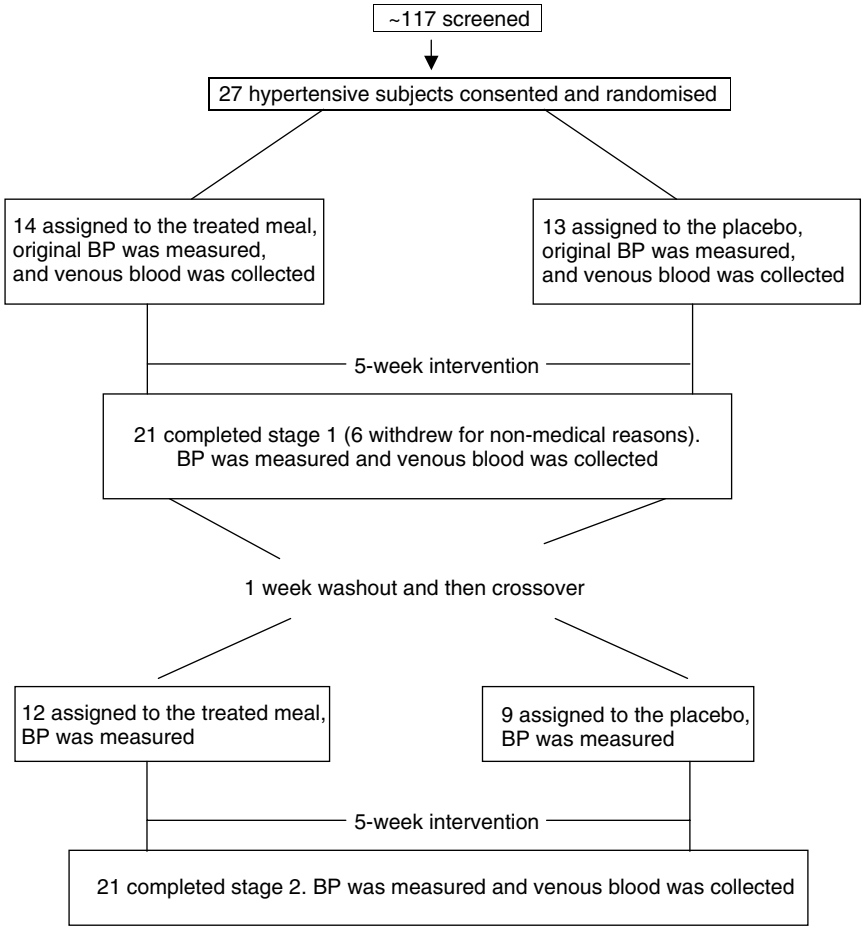

Figure 1. Summary of experimental design.

Statistical analysis

Effects of the treated meal and placebo on SHR blood pressure were assessed by one-way analysis of variance (ANOVA) followed by the *post hoc* Tukey test. A value of $P < 0.05$ was considered to be statistically significant at the same treatment time. The paired *t* test was used to compare blood pressure (SBP and DBP) readings before and after the feeding trial with treated meal or placebo, including originals *versus* stage 1 end, stage 2 beginning *versus* stage 2 end, and stage 1 end *versus* stage 2 beginning. A difference was considered statistically significant when $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$. One-way ANOVA followed by the *post hoc* Tukey test was used to compare either subjects' blood pressure (SBP and DBP) or serum lipid profiles and other biochemical measurements from venous blood collection among originals, treated meal intervention and placebo intervention, and a value of $P < 0.05$ was considered to be statistically significant during the feeding trial.

RESULTS AND DISCUSSION

Amount of dioscorin in treated meal

Figure 2 shows the ELISA standard curve of purified dioscorin (0.153, 0.313, 0.625 and 1.250 $\mu g\,mL^{-1}$) and the contents of dioscorin in the treated meal and placebo. A good correlation ($R^2 = 0.995$) was found between the different concentrations of dioscorin and the output readings of $A_{450\,nm}$ (Fig. 2). No dioscorin was detected in the placebo, but the treated meal contained 0.234 $\mu g\,mL^{-1}$ dioscorin (corresponding to 140 mg of dioscorin per packet). Our previous report[13] confirmed that purified dioscorin exhibited antihypertensive activity in SHRs. Therefore the instant foods with (treated meal) and without (placebo) lyophilised yam powder were used firstly to evaluate the antihypertensive effects on SHRs, after which the feeding trial in hypertensive subjects was conducted.

Effects of 4 week administration of treated meal and placebo on blood pressure of SHRs

Figure 3 shows the effects of the treated meal and placebo (2.5 g kg^{-1} SHR) on the SBP (Fig. 3(A)) and DBP (Fig. 3(B)) of SHRs over 4 weeks. One-way ANOVA followed by the *post hoc* Tukey test was performed for statistical analyses. It was found that the treated meal, but not the placebo, exhibited antihypertensive effects and led to significantly different SPB (Fig. 3(A), weeks 2, 3 and 4, $P < 0.05$ compared with control) and DBP (Fig. 3(B), week 2, $P < 0.05$ compared with control) in SHRs. The reduced SBP and DBP after treated meal administration (2.5 g kg^{-1} SHR) were 16.71, 18.50 and 24.08 mmHg and 15.64, 11.03 and 12.83 mmHg at weeks 2, 3 and 4 respectively compared with the control. The treated meal reduced SHR blood pressure more than the placebo, both SBP (Fig. 3(A), weeks 2, 3 and 4, $P < 0.05$, treated meal *versus* placebo) and DBP (Fig. 3(B), weeks 2 and 4, $P < 0.05$, treated meal *versus* placebo). The addition of lyophilised yam powder (treated meal) had clear antihypertensive effects on SHRs. Therefore the

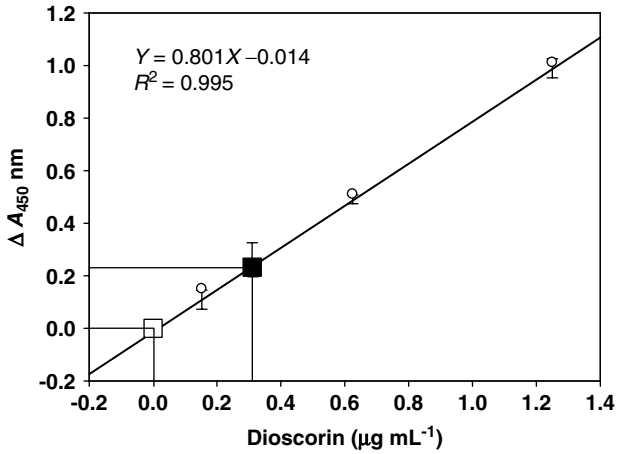

Figure 2. Amounts of dioscorin in treated meal and placebo as determined by ELISA using anti-dioscorin polyclonal antibody from mouse serum. Purified dioscorin (○: 0.153, 0.313, 0.625 and 1.250 μg mL^{-1}) was used to plot a standard curve to quantify dioscorin in the treated meal (■) and placebo (□). Means of triplicates were measured.

feeding trial was performed to evaluate the effects of the treated meal on hypertensive subjects.

Effects of treated meal and placebo on hypertensive subjects in feeding trial

Twenty-one subjects with an average age of 34.5 years completed the feeding trial, six of the original 27 subjects having withdrawn for non-medical reasons during stage 1 (Fig. 1). The effects of the treated meal and placebo on hypertensive subjects are shown in Fig. 4. The original SBP and DBP in the treated meal group were 132.83 ± 8.14 mmHg (O-treated meal_SBP) and 88.00±5.28 mmHg (O-treated meal_DBP) respectively, while those in the placebo group were 137.56 ± 8.85 mmHg (O-placebo_SBP) and 92.22 ± 9.28 mmHg (O-placebo_DBP) respectively. The original SBP and DBP in the treated meal and placebo groups (O-treated meal *versus* O-placebo) were not significantly different by the Student *t* test (data not shown). Figure 4(A) shows the changes in blood pressure for originals *versus* treated meal or placebo intervention in stage 1. After 5 week intervention in stage 1, the SBP and DBP in the treated meal group were 125.42 ± 7.02 mmHg (Treated meal_SBPstg1end) and 84.25 ± 7.04 mmHg (Treated meal_DBPstg1end) respectively, while those in the placebo group were 133.00 ± 12.39 mmHg (Placebo_SBPstg1end) and 88.33 ± 10.83 mmHg (Placebo_DBPstg1end) respectively. The reduced SBP and DBP after treated meal intervention in stage 1 were 7.42 and 3.75 mmHg respectively, significantly different from the original SBP (O-treated meal_SBP, $P < 0.001$) and DBP (O-treated meal_DBP, $P < 0.01$) by the paired *t* test. The reduced SBP and DBP after placebo intervention in stage 1 were 4.56 and 3.89 mmHg respectively, significantly different from the original SBP (O-placebo_SBP, $P < 0.05$) and DBP (O-placebo_DBP, $P < 0.05$) by the paired *t* test. The SBP reductions in the treated meal group were much higher than those in the placebo group (7.42 *vs* 4.56 mmHg). Although the DBP reduction after treated meal or placebo intervention in stage 1 was similar, the former differed more significantly ($P < 0.01$) from the original than the latter ($P < 0.05$). Figure 4(B) shows the changes in blood pressure for stage 2 beginning *versus* treated meal or placebo intervention in stage 2. After washout for 1 week, the SBP and DBP at stage 2 beginning in the placebo

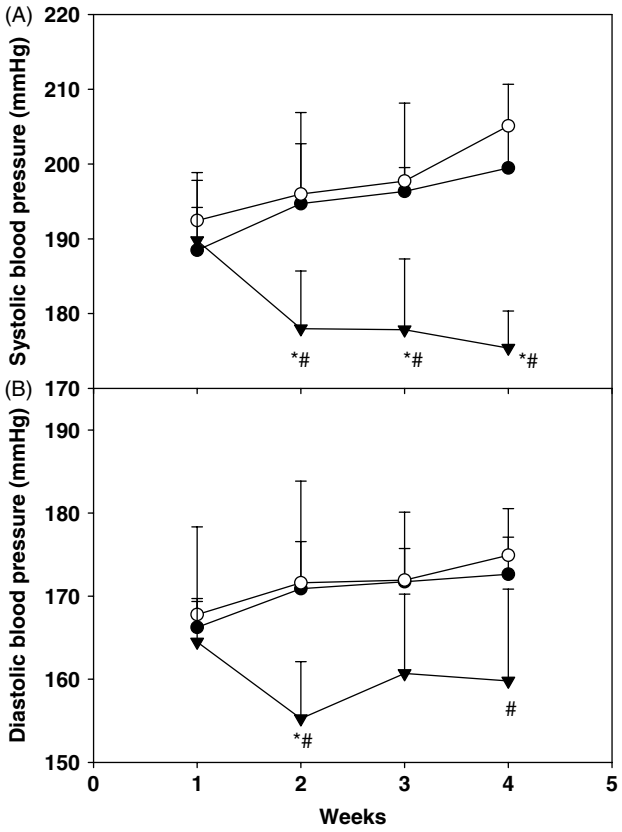

Figure 3. Effects of treated meal (▼) and placebo (○) at concentration of 2.5 g kg^{-1} SHR (suspended in 0.5 mL of water) on (A) systolic blood pressure and (B) diastolic blood pressure of SHRs ($n = 8$) over 4 weeks. Only 0.5 mL of water was administered orally to the control group (●). One-way ANOVA followed by the *post hoc* Tukey test was performed and a value of $P < 0.05$ was considered statistically significant. $^{*}P < 0.05$, treated meal or placebo *versus* control; $^{\#}P < 0.05$, treated meal *versus* placebo.

group were 127.58 ± 9.10 mmHg (Placebo_SBPstg2begin) and 84.42 ± 7.25 mmHg (Placebo_DBPstg2begin) respectively, while those in the treated meal group were 132.11 ± 15.32 mmHg (Treated meal_SBPstg2begin) and 87.44 ± 12.29 mmHg (Treated meal_DBPstg2begin) respectively. After 5 week intervention in stage 2, the SBP and DBP in the placebo group were 128.00 ± 7.73 mmHg (Placebo_SBPstg2end) and 85.25 ± 6.15 mmHg (Placebo_DBPstg2end) respectively, while those in the treated meal group were 132.67 ± 10.15 mmHg (Treated meal_SBPstg2end) and 86.44 ± 8.83 mmHg (Treated meal_DBPstg2end) respectively. Neither treated meal nor placebo intervention in stage 2 was significantly different from stage 2 beginning. It was also found that the SBP and DBP showed no significant change before and after 1 week washout (Fig. 4(C)). This meant that hypertensive subjects reached their stable blood pressure after stage 1 intervention with either treated meal or placebo and maintained it during the washout stage and subsequent 5 week crossover in the feeding trial. There have been several reports concerning statistical effects of the placebo group in nutrient supplement trials.[21–24] The instant food without lyophilised yam powder (placebo) was shown to lower blood pressure in the feeding trial (Fig. 4(A)), which might be attributed to a placebo effect. However, this effect was less than that caused by the treated meal. Figure 4(D) compares the SBP and DBP results among originals, treated meal intervention and placebo inter-

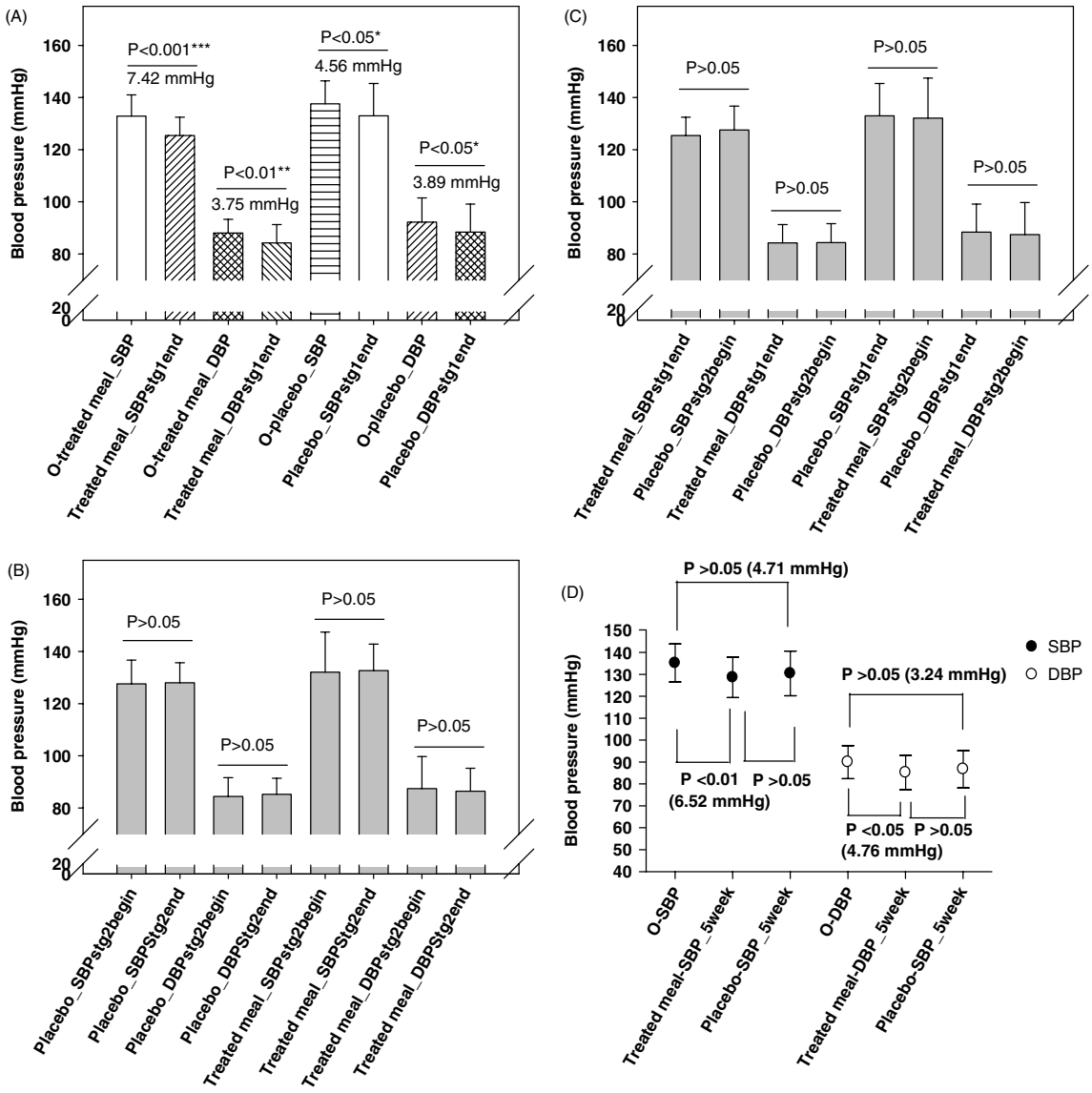

Figure 4. Effects of treated meal and placebo on systolic blood pressure (SBP) and diastolic blood pressure (DBP) of hypertensive subjects in feeding trial. The paired *t* test was used to compare changes in blood pressure (SBP and DBP) for (A) originals (O) *versus* treated meal or placebo after 5 week intervention in stage 1 (stg1end), (B) stage 2 beginning (stg2begin) *versus* treated meal or placebo after 5 week intervention in stage 2 (stg2end) and (C) 5 week intervention in stage 1 (stg1end) *versus* stage 2 beginning (stg2begin) of treated meal or placebo. A difference was considered statistically significant when $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$. One-way ANOVA followed by the *post hoc* Tukey test was used to compare changes in blood pressure (SBP and DBP) for (D) originals (O) *versus* treated meal or placebo and treated meal *versus* placebo after 5 week intervention. A value of $P < 0.05$ was considered statistically significant during the feeding trial.

vention by one-way ANOVA followed by the *post hoc* Tukey test. It was found that the SBP values of originals, treated meal intervention and placebo intervention were 134.86 ± 8.68 mmHg (O-SBP), 128.33 ± 9.17 mmHg (Treated meal-SBP_5Week) and 130.14 ± 10.17 mmHg (Placebo-SBP_5Week) respectively. The SBP after treated meal intervention, but not after placebo intervention, was significantly different ($P < 0.01$) from the original. It was found that the DBP values of originals, treated meal intervention and placebo intervention were 89.81 ± 7.47 mmHg (O-DBP), 85.05 ± 7.82 mmHg (Treated meal-DBP_5Week) and 86.57 ± 8.50 mmHg (Placebo-DBP_5Week) respectively. The DBP after treated meal intervention, but not after placebo intervention, was significantly different ($P < 0.05$) from the original. It was found that SBP and DBP reductions reached 6.52 and 4.76 mmHg respectively after treated meal intervention. Thus, according to the present results, intake of the instant food containing 140 mg of dioscorin over 5 weeks had a regulating effect on human blood pressure.

Effects of treated meal and placebo on serum lipid profiles and other biochemical measurements of cardiovascular risk in feeding trial

Venous blood was collected from each subject after feeding group assignment and at the end of the 5 week intervention with treated meal or placebo. Each venous blood sample was assayed for TC, TG, GOT, GPT, HDL and LDL as shown in Fig. 5. There were no significant differences ($P > 0.05$) among originals, treated meal intervention and placebo intervention by one-way ANOVA followed by the *post*

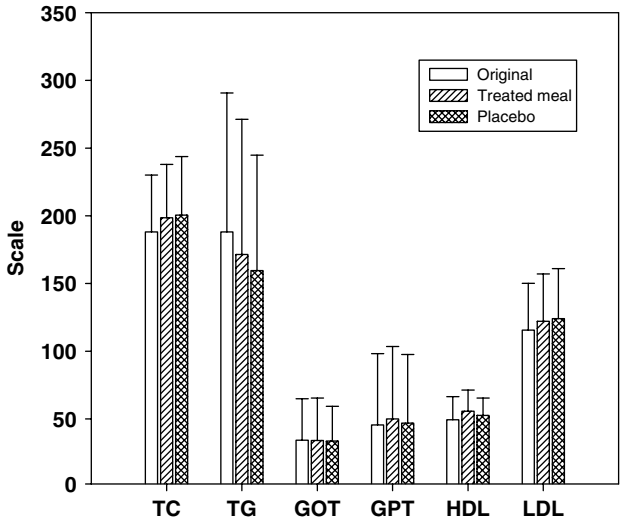

Figure 5. Effects of treated meal and placebo on serum lipid profiles and other biochemical measurements (TC, TG, GOT, GPT, HDL and LDL) in hypertensive subjects after 5 week intervention. One-way ANOVA followed by the *post hoc* Tukey test was performed and a difference was considered statistically significant when $P < 0.05$.

hoc Tukey test. This meant that the feeding trial did not appear to affect serum lipid profiles or other biochemical measurements of cardiovascular risk.

CONCLUSION

Intake of an instant food containing 140 mg of dioscorin over 5 weeks had a regulating effect on human blood pressure.

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