European Journal of Integrative Medicine 8 (2016) 654–660

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/eujim

Research paper

Efficacy and safety of oral *Antrodia cinnamomea* mycelium in mildly hypertensive adults: A randomized controlled pilot clinical study

Chin-Chu Chen[a,b,c], I-Chen Li[a], Ting-Wei Lin[a], Hsiao-Ling Chang[a], Wen-Hsin Lin[d], You-Cheng Shen[e,*]

[a] *Grape King Bio Ltd, Zhong-Li Dist., Taoyuan City 320, Taiwan*
[b] *Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan*
[c] *Department of Food Science, Nutrition and Nutraceutical Biotechnology, Shih Chien University, Taipei City, Taiwan*
[d] *School of Pharmacy, China Medical University, Taichung, Taiwan*
[e] *School of Health, Diet, and Industry Management, Chung Shan Medical University, Taichung, Taiwan*

ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form 25 May 2016
Accepted 4 June 2016

Keywords:
Antrodia cinnamomea mycelia
Blood pressure
Randomised clinical trial
Alternative treatment

ABSTRACT

Introduction: Evidence indicates that, in animal models, *Antrodia cinnamomea* (*AC*) mycelium is *in vitro* hypotensively active. Convincing evidence of the clinically relevant benefits of *AC* mycelium in humans, however, is unclear. Hence, this pilot randomised clinical trial was conducted to assess the effects of *AC* mycelium on blood pressure and other cardiovascular risk factors in patients with mild hypertension.
Methods: Forty-one patients with systolic blood pressure (SBP) between 130 and 179 mmHg or diastolic blood pressure (DBP) between 85 and 109 mmHg were randomised then treated with either *AC* mycelium or starch (placebo) for 8 weeks and were followed up for an additional 2 weeks.
Results: SBP in the patients treated with *AC* mycelium was significantly lower (144.86 ± 11.34 to 133.10 ± 10.90 mmHg; $p < 0.05$) than in patients treated with the placebo. DBP was also significantly ($p < 0.05$) lower (it fell from 96.19 ± 7.42 to 91.38 ± 7.56 mmHg) after 8 weeks of *AC* mycelium treatment. There were no significant changes in anthropometric, lipid profile, or biochemical parameters between the placebo- and *AC* mycelium-treated groups, except for reduced plasma renin activity after *AC* mycelium treatment.
Conclusions: There were neither adverse events nor abnormal laboratory findings throughout the study period, which suggested that *AC* mycelium significantly reduced mild hypertension; this might support the hypothesis that it is a safe alternative treatment for mild hypertension.
Trial registration: Chung Shan Medical University Hospital Internal Review Board approval number: CS11043; ClinicalTrial.gov registration number: NCT02532699.

1. Introduction

In 2013, the World Health Organization pointed out that hypertension is a global public health problem [1]. High blood pressure (BP) affects one in four adults worldwide and accounts for 40% of cardiovascular-related deaths [2]. Finding a viable preventative treatment is therefore crucial for helping to decrease the incidence and, therefore, the morbidity and mortality of the disease. In essence, practicing a healthy lifestyle and diet would be recommended as the first-line treatment in preventing hypertension. Mushrooms, for example, have significant medicinal value for diseases such as hypertension [3].

Antrodia cinnamomea (*AC*)(*niu-chang-chih* in Chinese) is a unique medicinal mushroom endemic to Taiwan. Primary investigations have revealed that *AC* is rich in numerous nutrients [4] – terpenoids, alkaloids, polysaccharides, proteins, and vitamins

Abbreviations: AC, *Antrodia cinnamomea*; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; BCRC, Bioresources Collection and Research Center; BMI, body mass index; BUN, blood urea nitrogen; BW, body weight; DBP, diastolic blood pressure; FBG, fasting blood glucose; GABA, gamma-aminobutyric acid; GSH, glutathione; GSH-Px, glutathione peroxidase; GSH-Rd, glutathione reductase; HDL, high-density lipoprotein; HPLC, high performance liquid chromatography; LDL, low-density lipoprotein; PRA, plasma renin activity; SBP, systolic blood pressure; SOD, Superoxide dismutase; SD, standard deviation; TBARS, thiobarbituric acid–reactive substances; TC, total cholesterol; TEAC, trolox equivalent antioxidant capacity; TG, triacylglycerol.

* Corresponding author at: School of Health, Diet, and Industry Management, Chung Shan Medical University, No. 110, Sec. 1, Jianguo N. Road, Taichung City 40201, Taiwan.
E-mail addresses: gkbioeng@grapeking.com.tw (C.-C. Chen), ichen.li@grapeking.com.tw (I.-C. Li), tingwei.lin@grapeking.com.tw (T.-W. Lin), hl.chang@grapeking.com.tw (H.-L. Chang), wslin@mail.cmu.edu.tw (W.-H. Lin), youcheng@csmu.edu.tw (Y.-C. Shen).

http://dx.doi.org/10.1016/j.eujim.2016.06.001
1876-3820/

– and has been used to treat various disorders, including hypertension [5]. Subsequently, a well-designed, preclinical study [6] was conducted to demonstrate that 10 mg/kg bw methanolic extract of a solid-state culture of *AC* was associated with a BP-reducing effect in spontaneously hypertensive rats, resulting in decreased systolic and diastolic BP. However, no study has yet used *AC* mycelium as a single agent on hypertensive patients to assess whether it is efficacious as a treatment. To investigate the cardiometabolic benefits of *AC* mycelium in humans, a randomized placebo-controlled trial was done.

2. Materials and methods

2.1. Patients and eligibility

This research protocol was approved by the institutional review board (IRB) of Chung Shan Medical University Hospital (Taichung, Taiwan; IRB No.: CS11043) and was assigned the National Clinical Trial (NCT) number: NCT02532699. Written informed consent from all participants in this study was obtained before their enrollment. Eligible participants were untreated hypertensive men or women between 20 and 80 years old with an SBP between 130 and 179 mmHg or a DBP between 85 and 109 mmHg measured in a sitting position. Participants were excluded if they had a history of major cardiovascular disease, severe liver dysfunction, insulin-dependent diabetes mellitus, or stroke. They were also excluded if they routinely consumed alcohol, were pregnant, or were unable to comprehend the study instructions.

2.2. Sample preparation and high-performance liquid chromatography (HPLC) analysis

AC (BCRC 35398) obtained from the Bioresources Collection and Research Center (BCRC) in the Food Industry Research and Development Institute (Hsinchu, Taiwan) was maintained on potato dextrose agar at 26 °C. After the AC had been incubated for 15 days, a mycelium agar block (0.5 cm^3) was removed, transferred to a 2-L Erlenmeyer flask containing 1.3 L of synthetic medium (1.0% glucose, 0.5% soybean powder, 0.5% peptone, and 0.01% $MgSO_4$, adjusted to pH 4.0) and incubated for 5 days at 28 °C on a rotary shaker (120 rpm). The fermentation process was then scaled up from a 2-L shake flask to 500-L fermenters for 5 days and 5-ton fermenters for 10 days. At the end of the fermentation process, the submerged fermentation culturing *AC* mycelium was harvested, lyophilized, ground into powder, and stored at 22 °C. The dry sample was then analyzed using high-performance liquid chromatography (HPLC) to evaluate the active adenosine, antrosterol, and gamma aminobutyric acid (GABA) content in the mycelium based on its dry weight. HPLC analyses of adenosine, antrosterol, and GABA in the *AC* powder were performed using the same procedures described elsewhere ([7–9]). The chemical compositional analysis, including calories, proteins, fats, carbohydrates, and sodium of three capsules containing freeze-dried mycelium were determined according to official Association Of Analytical Communities methods [10].

2.3. Study design

This 8-week double-blind randomized placebo-controlled parallel study with a 2-week follow-up period was performed in adult human subjects with mild hypertension [11], adhered to the CONSORT statement and carried out in accordance with The Code of Ethics of the World Medical Association. A random allocation sequence for assigning participants to one of the two study groups was created using a computerized random generator and concealed from the researchers to prevent selection and detection biases. Consenting eligible participants were treated for 8 weeks with three capsules per day containing either 420 mg of *AC* mycelium or 420 mg of starch (placebo) of similar appearance and taste. This dose of *AC* mycelium was chosen based on a pilot study which showed that it reduced SBP and DBP (data not shown). The participants were required to visit Chung Shan Medical University Hospital at baseline, every two weeks during the intervention period (8 weeks), and at follow-up 2 weeks after treatment had ended. During each study visit, SBP and DBP were recorded, fasting blood samples were collected, and anthropometric measurements were taken. Compliance was evaluated using a food diary and monitored using biweekly telephone calls.

2.4. Measurements

During all study visits, anthropometric measurements – weight, body mass index (BMI), and body fat percentage – were taken. BP tests were done according to World Health Organization guidelines [12] using a semi-automated digital blood pressure monitor (Rossmax InnoTek Corp., Taipei, Taiwan) and were supervised by a nurse. Participants were asked to avoid consuming caffeine or smoking 30 min before their appointment and had to rest in a seated position for 10 min before beginning the measurements. Fasting blood samples were collected for lipid panels, serum biochemistry, and oxidative values. For lipid panels and serum biochemistry analysis, the serum was separated from the blood after it has been centrifuged; it was then examined using an automated biochemistry analyzer (UniCel DxC 800 Synchron Clinical Systems; Beckman Coulter, California, USA), which included analyses of fasting blood glucose (FBG), triacylglycerol (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Plasma renin activity (PRA) was measured using a commercial ELISA kit (Ibl-America, Minneapolis, MN, USA). Six oxidative stress biomarkers [thiobarbituric acid–reactive substance (TBARS), trolox equivalent antioxidant capacity (TEAC), glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), and superoxide dismutase (SOD)] were used in whole blood with ethylenediamine tetraacetic acid (EDTA). Lipid peroxidation levels were expressed as the reaction product of malondialdehyde with TBARS [13]. The plasma total antioxidant capacity was estimated by GSH and TEAC based on the procedures described elsewhere [14]. Activity levels of GSH-Px and GSH-Rd in erythrocytes were assessed using methods described elsewhere [15,16], respectively. SOD activity was measured using commercially available assay kits (Randox, UK).

2.5. Sample size and statistical analysis

Based on the observations of a similar study [17], a change of 10 mmHg in SBP or DBP ± standard deviation (SD) = 13 was considered significant for determining the sample size: approximately 16 participants would be required ($\alpha = 0.05$ and $1\text{-}\beta = 0.8$). Because 20% losses from follow-up were considered possible, at least 20 participants per group had to be enrolled. Data are presented as mean ± SD. Paired *t* tests were used to compare variables before and after the intervention. Differences between groups were evaluated using Student's *t* test. SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Significance was set at $p < 0.05$.

3. Results

Of the 48 patients invited to participate in the study, 6 did not meet the inclusion criteria, and 1 withdrew for personal reasons

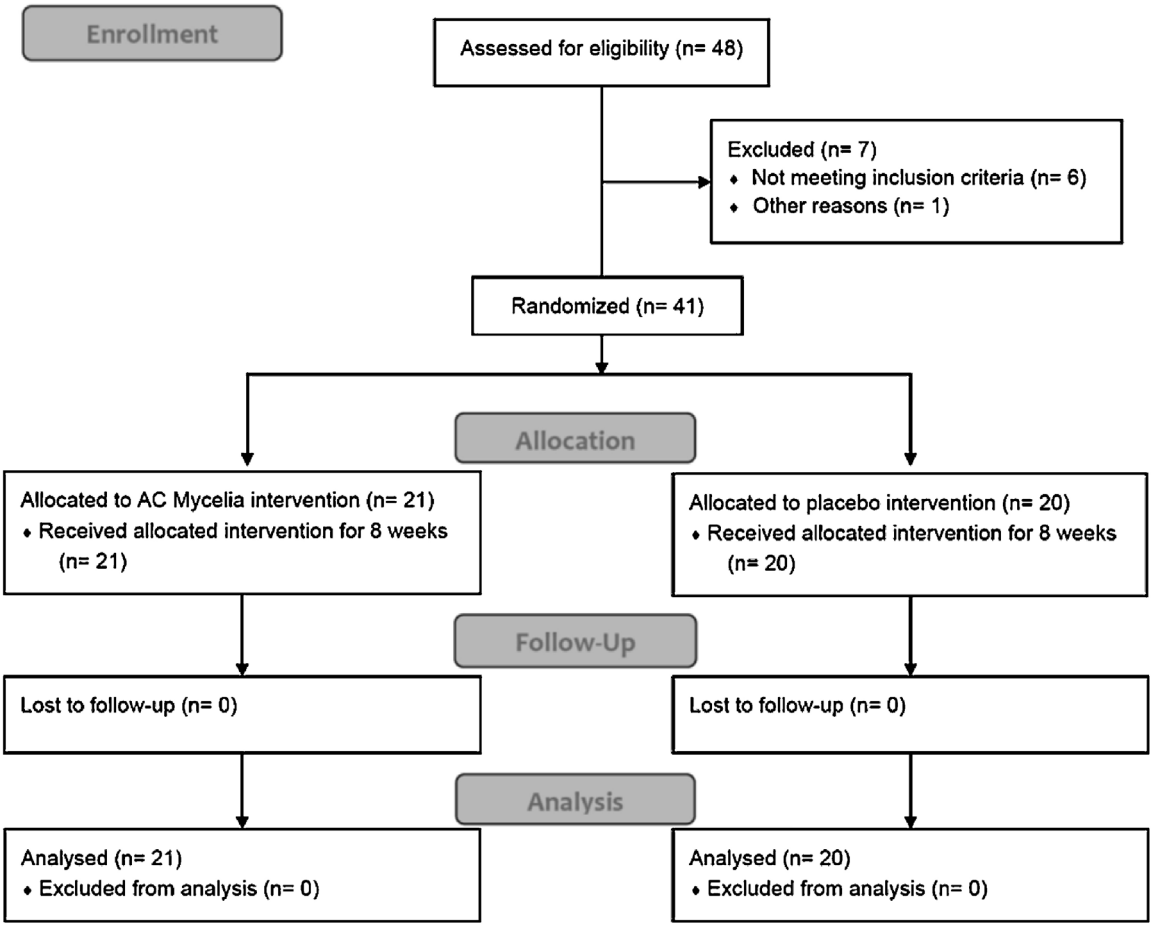

Fig. 1. CONSORT flow diagram.

unrelated to the trial. Participants were randomly assigned for *AC* mycelium ($n \geq 20$) or starch placebo treatment ($n \geq 20$); 21 participants from the *AC* mycelium group and 20 from the placebo group completed the study (Fig. 1).

The overall sample had a mean baseline SBP $\geq$ 140 mmHg and a mean baseline DBP $\geq$ 90 mmHg, a healthy fasting lipid profile with normal hepatic functions, and renal values within the normal ranges (Table 1) [18]. Differences between the mean age, mean body weight, mean body fat, mean BMI, and mean resting SBPs and DBPs were nonsignificant, and the biochemical indices – FBG, TG, TC, HDL, and LDL – were not significantly different.

3.1. Effects of AC mycelium on blood pressure

At baseline, the mean SBP/DBP in the *AC* mycelium-treated and placebo-treated groups were 144.86 ± 11.34/96.19 ± 7.42 mmHg and 145.70 ± 10.91/98.30 ± 6.91 mmHg, respectively (Fig. 2). There were no significant changes in either SBP (145.70 ± 10.91 to 142.20 ± 15.06 mmHg) or DBP (98.30 ± 6.91 to 96.05 ± 8.27 mmHg) values in the placebo group. The mean SBP in the *AC* mycelium-treated group was significantly lower (it fell by 11.76 mmHg), and continued to be so, even during the 10th week follow-up visit. Similarly, the mean DBP in the *AC* mycelium-treated group participants with elevated DBP at week 8 was 4.81 mmHg lower and during the 10th week follow-up visit was 5.0 mmHg lower compared with baseline, these differences at week 8 were significant ($p < 0.05$) in both the intergroup and intragroup comparisons.

Table 1
Baseline demographic, anthropometric, lipid profile and biochemical characteristics of mild hypertensive human subjects assigned to receive either AC mycelia or starch placebo.

Characteristics	Groups[a]	
	AC mycelia (n = 21)	Placebo (n = 20)
Male (n)	17	15
Female (n)	4	5
Age (y)	39.76 ± 10.07	40.15 ± 12.31
BW (kg)	77.22 ± 17.64	74.74 ± 14.35
Body fat (%)	28.14 ± 10.28	27.27 ± 5.6
BMI (kg/m$^{2)}$	26.66 ± 5.21	26.38 ± 4.18
SBP (mmHg)	144.86 ± 11.34	145.7 ± 10.91
DBP (mmHg)	96.19 ± 7.42	98.3 ± 6.91
FBG (mg/dl)	97.48 ± 10.38	100.6 ± 11.83
TG (mg/dl)	171.71 ± 110.03	172.85 ± 97.24
TC (mg/dl)	203.9 ± 31.68	196.45 ± 30.14
HDL (mg/dl)	43.84 ± 7.81	43.65 ± 7.57
LDL (mg/dl)	121.95 ± 27.24	119.46 ± 29.82
Creatinine (mg/dl)	0.88 ± 0.2	0.88 ± 0.18
BUN (mg/dl)	9.47 ± 0.2	10.4 ± 2.8
AST (IU/l)	24.38 ± 6.52	25.4 ± 9.19
ALT (IU/l)	29.48 ± 19.26	30.2 ± 21.96

Values are presented as mean ± SD. BMI: Body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; TG: triacylglycerol; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

[a] No significant between-group differences were found.

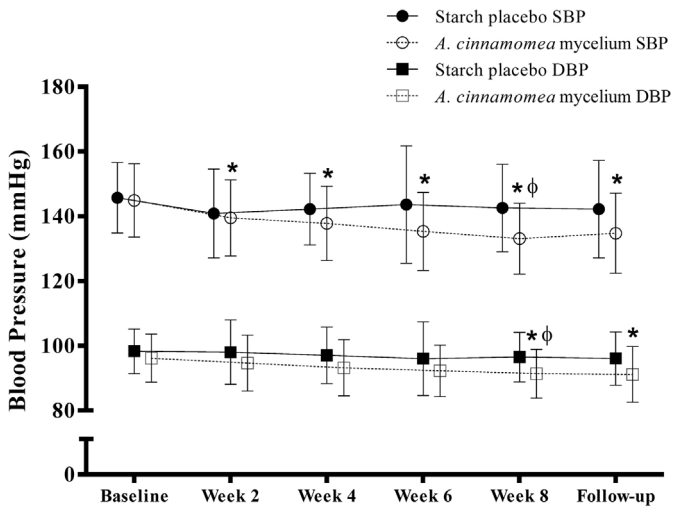

Fig. 2. The mean SBP and DBP in patients from the treatment group (n = 21) and the placebo group (n = 20) during 10 weeks of study. Data were expressed as mean ± SD. *Statistically significant ($p < 0.05$) when compared to baseline values. $^{\Phi}$Statistically significant ($p < 0.05$) when compared to placebo group. SBP: systolic blood pressure; DBP: diastolic blood pressure.

3.2. Effects of AC mycelium on anthropometric, lipid profile, and blood biochemical values

Changes in body fat values were not statistically significant (Table 2). In the *AC* mycelium-treated group, body weight decreased from 77.22 ± 17.64 to 76.1 ± 16.96 kg with a parallel reduction in BMI (26.66 ± 5.21 to 26.28 ± 5.01) nonsignificant compared with baseline or the placebo group.

TC levels in the lipid profile were significantly ($p < 0.05$) lower (203.90 ± 31.68 to 190.57 ± 31.28) in the *AC* mycelium-treated group, but not in the placebo group (196.45 ± 30.14 to 199.05 ± 32.96). Changes in other parameters – TG, HDL, and LDL levels – were not significantly different in either group (Table 2).

The participants' mean FBG continuously fell in both groups (Table 2). However, all FBG values were within the normal range [18]. Creatinine, BUN, and ALT levels were not significantly different between the two groups (Table 2), but AST levels were significantly higher after 8 weeks of *AC* mycelium treatment, and PRA significantly ($p < 0.05$) lower than in the control group (Table 2).

3.3. Effects of AC mycelium on oxidative stress status and antioxidant enzyme activities

The antioxidant capacity of the *AC* mycelium-treated and placebo groups was measured, using the TEAC assays, as scavenging free radicals. There was no significant difference in this activity between the two groups (Table 3). However, after 8 weeks of *AC* mycelium treatment, there was a significant ($p < 0.05$) decrease in the TBARS level (Table 3). *AC* mycelium treatment was also linked with a significant ($p < 0.05$), compared with the baseline, decrease in GSH-Px and GSH-Rd activities during week 8 (Table 3).

3.4. Chemical composition and major active compounds in AC mycelium

Recently, multiple components of food or herbs have been analyzed for their authenticity, consistency, and stability. The chemical composition analysis of freeze-dried *AC* mycelium showed 3.83% moisture, 35.54% ash, 28.70% crude protein, 9.56% crude fat, and 22.37% carbohydrate. There were symmetrical peaks for adenosine (Fig. 1A; bottom), antrosterol (Fig. 1B; bottom), GABA (Fig. 1C; bottom), and their standards (top). The retention times of adenosine, antrosterol, and GABA were 9.367, 9.600, and 7.800 min, respectively. The quantification was done using peak area ratios of the compound to internal standards. The levels of adenosine, antrosterol, and GABA in *AC* mycelium were 1.18, 4.52, and 5.15 mg/g, respectively (Fig. 3).

3.5. Safety and tolerability

Safety of *AC* mycelium treatment was assessed using metabolic variables. Liver and kidney functions were not affected, as shown by various blood serum parameters. Throughout the 8-week

Table 2
Effects of dietary AC mycelia on anthropometric, lipid profile and biochemical characteristics of mild hypertensive human subjects over 8 weeks.

Parameters	Groups			
	A. cinnamomea mycelia (n = 21)		Placebo (n = 20)	
	Baseline	End of study	Baseline	End of study
Anthropometric values				
BW (kg)	77.22 ± 17.64	76.1 ± 16.96[a]	74.74 ± 14.35	73.66 ± 14.33*
Body fat (%)	28.14 ± 10.28	27.55 ± 10.91	27.27 ± 5.6	27.02 ± 6.85
BMI (kg/m^2)	26.66 ± 5.21	26.28 ± 5.01[a]	26.38 ± 4.18	25.97 ± 4.06*
Lipid profiles				
TG (mg/dl)	171.71 ± 110.03	157.05 ± 98.61	172.85 ± 97.24	192.1 ± 149.61
TC (mg/dl)	203.9 ± 31.68	190.57 ± 31.28[a]	196.45 ± 30.14	199.05 ± 32.96
HDL (mg/dl)	43.84 ± 7.81	42.9 ± 7.36	43.65 ± 7.57	44.55 ± 7.37
LDL (mg/dl)	121.95 ± 27.24	116.15 ± 27.21	119.46 ± 29.82	119.21 ± 31.7
Blood biochemical values				
FBG (mg/dl)	97.48 ± 10.38	89.48 ± 8.15[a]	100.6 ± 11.83	90.3 ± 7.09*
Creatinine (mg/dl)	0.88 ± 0.2	0.83 ± 0.12	0.88 ± 0.18	0.85 ± 0.19
BUN (mg/dl)	9.47 ± 2.2	10.24 ± 2.21	10.4 ± 2.8	11.2 ± 2.86
AST (IU/l)	24.38 ± 6.52	21.05 ± 5.10[a]	25.4 ± 9.19	25.85 ± 14.07
ALT (IU/l)	29.48 ± 19.26	28.9 ± 21	30.2 ± 21.96	33 ± 28.79
PRA (ng/ml h)	2.57 ± 0.88	1.84 ± 0.66[a,b]	2.78 ± 0.89	2.84 ± 0.74

Values are presented as mean ± SD. BW: Body weight; BMI: Body mass index; TG: triglyceride; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; FBG: fasting blood glucose; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; PRA: plasma renin activity.
[a] Statistically significant ($p < 0.05$) when compared to baseline values.
[b] Statistically significant ($p < 0.05$) when compared to placebo group.

Table 3
Effects of dietary AC mycelia on oxidative stress status and antioxidant enzyme activities of mild hypertensive human subjects over 8 weeks.

Parameters	Groups			
	A. cinnamomea mycelia (n = 21)		Placebo (n = 20)	
	Baseline	End of study	Baseline	End of study
Oxidative stress status				
TEAC (μmol Trolox equivalent)/mL	8.87 ± 0.58	8.68 ± 0.67	8.82 ± 0.84	8.6 ± 0.46
TBARS μmol/mL	1.34 ± 0.6	0.83 ± 0.53[a]	1.55 ± 0.54	1.34 ± 0.89
Antioxidant enzyme activities				
GSH (μM)	1.69 ± 0.59	1.73 ± 0.4[b]	1.39 ± 0.54	1.06 ± 0.63
GSH-Px (IU/g Hb)	25.93 ± 6.83	34.21 ± 9.53[a,b]	26.16 ± 6.45	24.88 ± 6.77
GSH-Rd	4.34 ± 2.7	7.46 ± 3.05[a,b]	4.26 ± 2.82	4.23 ± 2.57
SOD (IU/g Hb)	1778.25 ± 375.14	1807.4 ± 296.58	1823.74 ± 362.32	1727.26 ± 345.93

Values are presented as mean ± SD. TEAC: trolox equivalent antioxidant capacity; TBARS: thiobarbituric acid reactive substances; GSH: glutathione; GSH-Px: glutathione peroxidase; GSH-Rd: glutathione reductase; SOD: superoxide dismutase.
[a] Statistically significant ($p < 0.05$) when compared to baseline values.
[b] Statistically significant ($p < 0.05$) when compared to placebo group.

intervention, there were no adverse events nor any other complications reported. All laboratory safety parameters remained within normal reference limits.

4. Discussion

There are few published reports with an accurate assessment of the bioactive components on submerged culture of *AC* mycelium. This is the first double-blinded, randomized controlled clinical trial to investigate the effects and safety of 8 weeks of treatment with three daily capsules of *AC* mycelium. We found that *AC* mycelium treatment lowered mean SBP (significant compared with the placebo group) and DBP levels to 10.01 and 4.81 mmHg, respectively, from baseline. This was associated with a 16–40% reduction in the risk of cardiovascular events [19,20]. Furthermore, this outcome was expected in light of the results from a previous animal study using spontaneously hypertensive rats [6]; thus, indicating the effectiveness of *AC* mycelium in controlling BP.

The mechanism that *AC* mycelium uses to reduce BP might be associated with the renin-angiotension system (RAS), a regulatory cascade initiated by the secretion of renin, which is crucial for the homeostatic control of arterial pressure [21]. Dysregulation of the RAS has been associated with hypertension. Compared with the placebo, *AC* mycelium significantly reduced PRA by a maximum of 36% on week 8, which suggested that *AC* mycelium is a potent inhibitor of renin, and that its bioavailability is sufficient to lower BP after short-term oral treatment. Thus, it is reasonable to assume that the decline in PRA is linked to the downstream inhibition of angiotensin II formation, which further decreases the sympathetic outflow that leads to hypertension.

In terms of anthropometric values, BW and BMI decreased from pre- to post- intervention in both groups. These reductions might have been the result of the participants' awareness of higher-energy foods by keeping a detailed dietary diary. A pre-clinical study [22] of a rat model confirmed that *AC* mycelium reduced serum TC, TG, and LDL. The present study found a significant reduction of TC levels, but not TG or LDL levels, after 8 weeks of *AC* mycelium treatment. In an animal models [23], after 5 weeks of *AC* mycelium treament, FBG levels in rats with streptozotocin-induced diabetes were significantly reduced. Consistently, the results of the present study showed that *AC* mycelium treatment considerably lowered the overall mean FBG, which supports its use for treating humans with diabetes. However, mean FBG levels in the placebo group also fell after 8 weeks of treament with potato starch. These attenuated blood sugar levels might contribute to the formation of starch resistant to digestive enzymes and has been shown to help maintain glycemic health by improving insulin secretion [24].

In addition to its therapeutic value for treating hypertension, hypercholesterolemia, and hyperglycemia, *AC* mycelium was therapeutic for patients with mildly elevated hypertension: an animal study [25] reported that antrosterol, a bioactive aompound in *AC* mycelium significantly prevented the elevation of AST and ALT in mice with carbon tetrachloride-induced liver damage. According to biochemical indices, antrosterol-containing *AC* mycelium treatment significantly reduced AST levels. However, there was no significant difference in ALT levels. In the present study, after 8 weeks of *AC* mycelium treatment, neither AST nor ALT levels increased and they remained within the normal range [18]. The implication is that *AC* mycelium is not hepatotoxic, and that, in human adults, it is feasible, safe and well-tolerated.

One study [26] of this Chinese medicine hypothesized that the beneficial effects of *AC* mycelium were related to its abilities to mediate antioxidants, scavenge free radicals, and inhibit inflammatory mediators. In the present study, however, *AC* mycelium-treated group had no significant changes in TEAC, a biomarker of the total antioxidant capacity of peripheral blood plasma [27]. In contrast, TBARS levels, an indicator of lipid peroxidation and oxidative stress, were significantly lower in the *AC* mycelium-treated group than in the placebo group. Increases in GSH levels and GSH-Px and GSH-Rd activities were significant, increases in SOD activity were not. This suggested that *AC* mycelium might contribute to the suppression of inflammatory activity that is ultimately associated with anti-hypertension effects.

Other studies [28,29] have shown that GABA-enriched (the GABA equivalent of 10 mg/day) or adenosine-enriched foods significantly reduced BP (at least 11.1/1 mmHg for SBP and 7.4/0 mmHg for DBP, respectively, during week 8) in randomized controlled trials. Based on our HPLC analysis, treatment with three capsules of *AC* mycelium increased the GABA and adenosine intakes to 15.15 mg/day and 3.54 mg/day, respectively. In the present study, after 8 weeks, the *AC* mycelium treatment lowered SBP by approximately 10 mmHg and DBP by 5 mmHg, which indicated that the hypotensive effect can be attributed to these compounds.

Furthermore, tests on the toxicity of *AC* mycelium were done *in vitro* and *in vivo*. Findings from various toxicity assessments [30,31] indicated that *AC* mycelium from liquid fermentation at the highest dose of 3000 mg/kg BW/day are safe. Considering that no adverse events were reported in the present study, and that all laboratory safety parameters remained within normal limits, *AC* mycelium might be safe for adult therapy. However, larger sized samples and

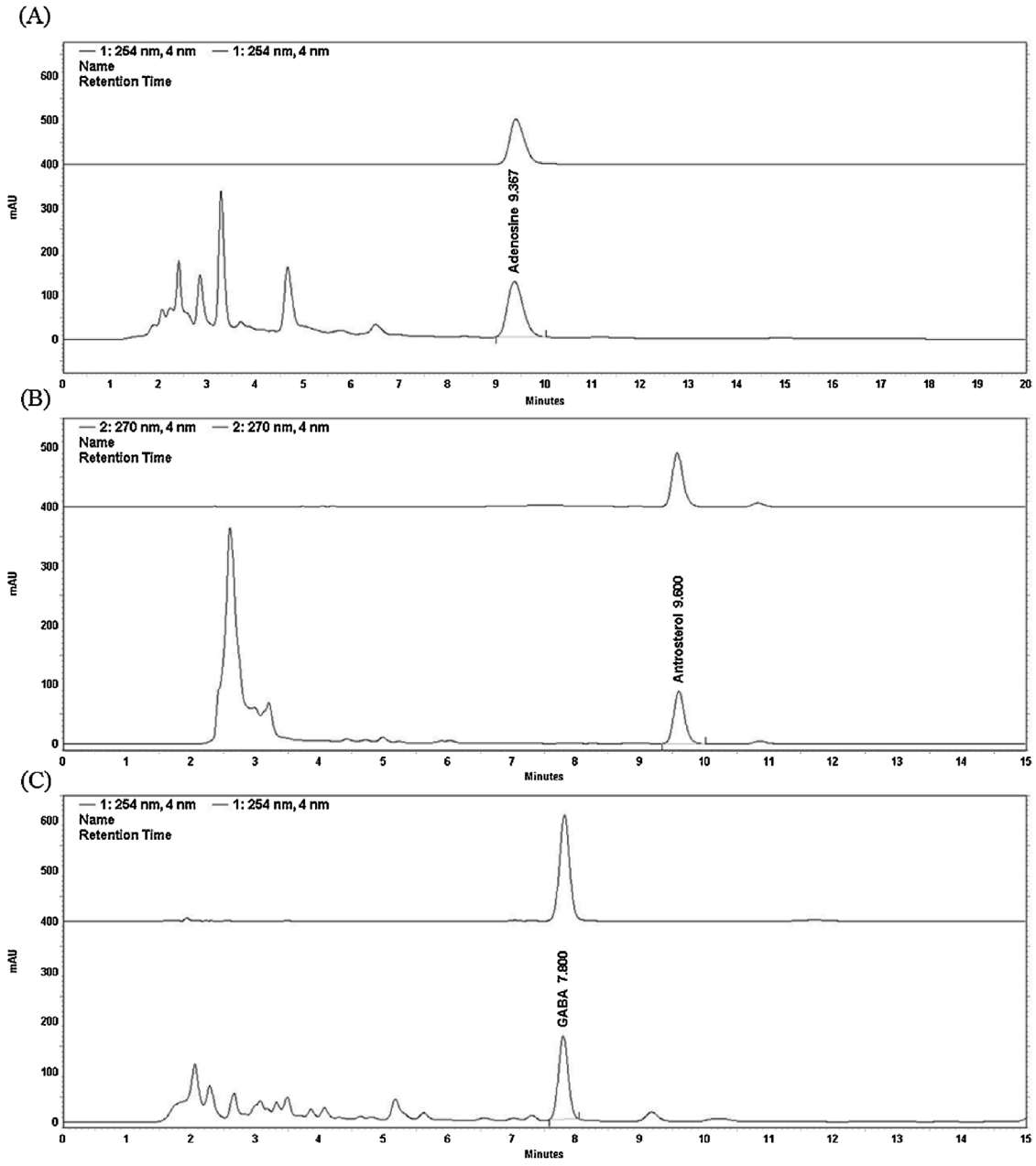

Fig. 3. HPLC chromatograms obtained from analyses of (A) adenosine, (B) antrosterolm and (C) GABA in AC mycelium. Top: standards; Bottom: samples; GABA: gamma-aminobutyric acid.

longer term trials are needed in future studies to provide greater insights into the effects of *AC* mycelium on the cardiovascular system.

5. Conclusion

This is the first clinical investigation that explores the anti-hypertensive effect of fermented *AC* mycelium in patients with mild hypertension. Overall, *AC* mycelium treatment for 8 weeks reduced mean SBP and DBP by inhibiting PRA, which is linked to the downstream inhibition of angiotensin II formation. In turn, sympathetic outflow is decreased, which leads to hypertension. In addition to its blood pressure-lowering properties, *AC* mycelium also significantly reduces oxidative stress. Furthermore, none of our participants experienced any adverse events during the study, which suggested that *AC* mycelium is a safe alternative treatment for high blood pressure in mildly hypertensives but currently unmedicated patients.

Conflict of interest

All authors declared no conflict of interest.

Authors' contribution

I-Chen Li designed the experiment, analyzed the clinical data, and wrote the first draft of the manuscript. Ting-Wei Lin and Hsiao-Ling Chang provided GABA, adenosine, and antrosterol-containing *AC* mycelium for healthy controls and mildly hypertensive patients. Wen-Hsin Lin assisted with the statistical analyses. Chin-Chu Chen and You-Cheng Shen coordinated the study, supervised the treatment, collected clinical data, and helped write the manuscript.

Financial support

None. This research was not supported by any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgment

We thank Hsin-Yun Yang for editing the manuscript.

References

[1] W.H. Organization, A global brief on hypertension: silent killer, Global Public Health Crisis, World Health Organization, Geneva, Switzerland, 2013.
[2] A.L. Martiniuk, C.M. Lee, C.M. Lawes, H. Ueshima, I. Suh, T.H. Lam, D. Gu, V. Feigin, K. Jamrozik, T. Ohkubo, M. Woodward, Hypertension: its prevalence and population-attributable fraction for mortality from cardiovascular disease in the Asia-Pacific region, J. Hypertens. 25 (1) (2007) 73–79.
[3] N.F. Mohamed Yahaya, M.A. Rahman, N. Abdullah, Therapeutic potential of mushrooms in preventing and ameliorating hypertension, Trends Food Sci. Technol. 39 (2) (2014) 104–115.
[4] M. Geethangili, Y.M. Tzeng, Review of pharmacological effects of antrodia camphorata and its bioactive compounds, Evidence-Based Complem. Altern. Med. eCAM 2011 (2011) 212641.
[5] Z.H. Ao, Z.H. Xu, Z.M. Lu, H.Y. Xu, X.M. Zhang, W.F. Dou, Niuchangchih (Antrodia camphorata) and its potential in treating liver diseases, J. Ethnopharmacol. 121 (2) (2009) 194–212.
[6] D.Z. Liu, Y.C. Liang, S.Y. Lin, Y.S. Lin, W.C. Wu, W.C. Hou, C.H. Su, Antihypertensive activities of a solid-state culture of Taiwanofungus camphoratus (Chang-chih) in spontaneously hypertensive rats, Biosci. Biotechnol. Biochem. 71 (1) (2007) 23–30.
[7] C.Y. Chang, M.Y. Lue, T.M. Pan, Determination of adenosine, cordycepin and ergosterol contents in cultivated *Antrodia camphorate* by HPLC method, J. Food Drug Anal. 13 (4) (2005) 338–342.
[8] W.G. Gallardo, A. Hagiwara, K. Hara, K. Soyano, T.W. Snell, GABA 5-HT and amino acids in the rotifers Brachionus plicatilis and Brachionus rotundiformis, comparative biochemistry and physiology, Part A Mol. Integr. Physiol. 127 (3) (2000) 301–307.
[9] J.-S. Lee, C.-M. Ma, M. Hattori, Quantification of ergosterol and ergosterol peroxide in several medicinal fungi by high performance liquid chromatography monitored with a diode array detection-atmospheric pressure chemical ionization-ion trap mass spectrometer, J. Tradit. Med. 25 (1) (2008) 18–23.
[10] A. International, Official Methods of Analysis of AOAC International, AOAC International, Gaithersburg, Md, 1995.
[11] R.H. Fagard, Do we need drug therapy to manage mild hypertension in the elderly? Drugs Aging 21 (1) (2004) 1–6.
[12] J. Chalmers, S. MacMahon, G. Mancia, J. Whitworth, L. Beilin, L. Hansson, B. Neal, A. Rodgers, C. Ni Mhurchu, T. Clark, World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. Guidelines sub-committee of the World Health Organization, Clin. Exp. Hypertens. 21 (5–6) (1999) 1009–1060.
[13] H.H. Draper, M. Hadley, Malondialdehyde determination as index of lipid peroxidation, Methods Enzymol. 186 (1990) 421–431.
[14] Y.Y. Chang, C.H. Chou, C.H. Chiu, K.T. Yang, Y.L. Lin, W.L. Weng, Y.C. Chen, Preventive effects of taurine on development of hepatic steatosis induced by a high-fat/cholesterol dietary habit, J. Agric. Food Chem. 59 (1) (2011) 450–457.
[15] L.R. Barlow-Walden, R.J. Reiter, M. Abe, M. Pablos, A. Menendez-Pelaez, L.D. Chen, B. Poeggeler, Melatonin stimulates brain glutathione peroxidase activity, Neurochem. Int. 26 (5) (1995) 497–502.
[16] A. Wendel, Glutathione peroxidase, Methods Enzymol. 77 (1981) 325–333.
[17] S. Mizuno, K. Matsuura, T. Gotou, S. Nishimura, O. Kajimoto, M. Yabune, Y. Kajimoto, N. Yamamoto, Antihypertensive effect of casein hydrolysate in a placebo-controlled study in subjects with high-normal blood pressure and mild hypertension, Br. J. Nutr. 94 (1) (2005) 84–91.
[18] W.M. Haynes, CRC Handbook of Chemistry and Physics, 95 edition, CRC Press, 2014.
[19] S. Lewington, R. Clarke, N. Qizilbash, R. Peto, R. Collins, Collaboration Prospective Studies, Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies, Lancet 360 (9349) (2002) 1903–1913.
[20] G.T. McInnes, Lowering blood pressure for cardiovascular risk reduction, J. Hypertens. 23 (1) (2005) S3–8.
[21] S.A. Atlas, The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition, J. Manag. Care Pharm. JMCP 13 (8) (2007) 9–20.
[22] J. Qi, Y. Tao, J. Zhang, J. Fu, Effect of Antrodia cinnamomea on gene expression related to aortal endothelial injury in rats with hyperlipidemia, Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi 39 (9) (2014) 1670–1674.
[23] Jong-Wook Shin, S.-I. Lee, S.-D. Kim, Effect of acetic acid fermented juice prepared using submerged culture media of Antrodia Camphorata Mycelium on blood glucose and lipid profiles of rats in which diabetes was induced with streptozotocin, Korean J. Food Preserv. 15 (5) (2008) 725–730.
[24] C.L. Bodinham, L. Smith, E.L. Thomas, J.D. Bell, J.R. Swann, A. Costabile, D. Russell-Jones, A.M. Umpleby, M.D. Robertson, Efficacy of increased resistant starch consumption in human type 2 diabetes, Endocr. Connect. 3 (2) (2014) 75–84.
[25] G.-J. Huang, J.-S. Deng, S.-S. Huang, Y.-Y. Shao, C.-C. Chen, Y.-H. Kuo, Protective effect of antrosterol from Antrodia camphorata submerged whole broth against carbon tetrachloride-induced acute liver injury in mice, Food Chem. 132 (2) (2012) 709–716.
[26] G. Hsiao, M.Y. Shen, K.H. Lin, M.H. Lan, L.Y. Wu, D.S. Chou, C.H. Lin, C.H. Su, J.R. Sheu, Antioxidative and hepatoprotective effects of Antrodia camphorata extract, J. Agric. Food Chem. 51 (11) (2003) 3302–3308.
[27] N.J. Miller, C. Rice-Evans, M.J. Davies, V. Gopinathan, A. Milner, A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, Clin. Sci. 84 (4) (1993) 407–412.
[28] K. Inoue, T. Shirai, H. Ochiai, M. Kasao, K. Hayakawa, M. Kimura, H. Sansawa, Blood-pressure-lowering effect of a novel fermented milk containing [gamma]-aminobutyric acid (GABA) in mild hypertensives, Eur. J. Clin. Nutr. 57 (3) (2003) 490–495.
[29] X.J. Xiong, P.Q. Wang, S.J. Li, X.K. Li, Y.Q. Zhang, J. Wang, Garlic for hypertension: a systematic review and meta-analysis of randomized controlled trials, Phytomedicine 22 (3) (2015) 352–361.
[30] T.I. Chen, C.C. Chen, T.W. Lin, Y.T. Tsai, M.K. Nam, A 90-day subchronic toxicological assessment of Antrodia cinnamomea in Sprague-Dawley rats, Food Chem. Toxicol. 49 (2) (2011) 429–433.
[31] T.I. Chen, C.W. Chen, T.W. Lin, D.S. Wang, C.C. Chen, Developmental toxicity assessment of medicinal mushroom Antrodia cinnamomea T. T. Chang et W. N. Chou (higher Basidiomycetes) submerged culture mycelium in rats, Int. J. Med. Mushrooms 13 (6) (2011) 505–511.