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Supercritical Fluid CO_2 Extracted-Chinese Yam (*Dioscorea polystachya*) Extracts on the Hypertension Regulation in Rats

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Keywords:

Supercritical Fluid CO_2 Extraction; Chinese Yam; Hypertension Regulation; In Vivo

[#]Author Contributions:

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Abbreviations:

ANOVA: Analysis Of Variance; BUN: Blood Urea Nitrogen; BW: Body Weight; CRE: Creatinine; CO_2: Carbon Dioxide; DBP: Diastolic Blood Pressure; IACUC: Institutional Animal Care And Utilization Committee; p.o: Oral Administration; GOT: Glutamic-Oxaloacetic Transaminase; GPT: Glutamic-Pyruvic Transaminase; MBP: Mean Blood Pressure; SBP: Systolic Blood Pressure; SEM: Standard Error Of The Mean; SFE: Supercritical Fluid Extraction; SHRs: Spontaneously Hypertensive Rats; WKY: Wistar Kyoto

1. Abstract

The aim of this research was the application of the supercritical fluid carbon dioxide (CO_2) extraction to extract Chinese yam (*Dioscorea polystachya*) and evaluated the efficacy on the hypertension regulation in rats. According to the literatures, Chinese yam is a perennial climbing vine, native to China and East Asia. Chinese yam is one of the important tropical plants in Taiwan because it's high nutritional values with high content of bioactive compounds that improve human health. This species of Chinese yam is unique as the tubers can be eaten raw. The edible tubers of Chinese yam are cultivated largely in Asia and sometimes used in alternative medicine. In this study, Chinese yam was extracted by the supercritical fluid CO_2 extraction. The extraction rate of Chinese yam was 1.0%. Later, LC-MS/MS and spectrophotometer were used to detect choline, diosgenin, allantoin, and lecithin contents of Chinese yam extracts. The choline content of Chinese yam extracts was 812.61 μg/g; the diosgenin content of Chinese yam extracts was 0.13 μg/g; the allantoin content of Chinese yam extracts was 5743.42 μg/g; the lecithin content of Chinese yam extracts was 3.8 g/100 g. Additionally, Chinese yam extracts were used to perform the hypertension regulation test *in vivo*. The Chinese yam extracts were orally administered to the spontaneously hypertensive rats

(SHRs) to evaluate the efficacy on regulating hypertension. *In vivo* results were showed that the body weight in each group was not significantly different with or without the Chinese yam extracts. The mean blood pressure (MBP), systolic blood pressure (SBP), and diastolic blood pressure (DBP) of SHRs were monitored once two weeks. Data were presented that MBP was decrease at 6_{th} week administration. After oral administration with the Chinese yam extracts for 6 weeks, the trend of the decrease MBP of SHR can be found that compared to the beginning of this experiment. Additionally, the liver and kidney functional indexes were all at the safety level after 6 weeks administration with the Chinese yam extracts. Taken these results together, the supercritical fluid CO_2 extracted-Chinese yam (*Dioscorea polystachya*) extracts has a potential ability to regulate hypertension.

2. Introduction

At present, a major global health concern is hypertension [1]. Hypertension has affected many people (about 73 million) in USA [1]. In 2025, 1.56 billion people will be suffered with hypertension in the world [1]. In Taiwan, the hypertension is the 8^{th} leading cause of death. Therefore, a lot of anti-hypertensive drugs have been researched and developed such as nifedipine, captopril, fosinopril, lisinopril etc. Although these drugs are effective in the regulation of hypertension, however, side-effects are often found [2-5].

Chinese yam (*Dioscorea polystachya*) was native to China, Korea, the Kuril Islands and Taiwan. At present, this plant grows throughout East Asia included Japan, Philippines, and Vietnam etc. Whereas most edible yams are tropical species, Chinese yam could be cultivated widely in Europe, the south-east USA, Uruguay and Western Himalaya, and other temperate regions in order to take advantage of its nutritional and bioactive properties. Moreover, Chinese Yam are distributed almost ubiquitously in the tropics and subtropics. They are cultivated as food crops in Africa, Asia, parts of South America and the Caribbean, and in the South Pacific islands [6-8].

Chinese yam has been cultivated as a food crop for centuries in East Asia and is a widely-used ingredient in traditional Chinese medicine. Chinese yam has been demonstrated that they possessed the high content of bioactive compounds. Their tubers are rich in nutrients contain bioactive metabolites such as resistant starches, steroidal sapogenins (like diosgenin), the storage protein dioscorin, and mucilage polysaccharides. These health-promoting products can help to prevent cardiovascular disease, diabetes, and disorders of the gut micro biome [9].

While the proper use of Chinese yam as health function additives could generate economic gains for agriculture. Currently, diet and lifestyle have been demonstrated that they also play a significant role in the hypertension regulation [1]. Lots of nature functional plants-derived extracts with anti-hypertensive activity have been discovered. Generally, these nature functional plants-derived extracts with anti-hypertensive activity are not toxic [2, 10]. Hence, the extracts were obtained from Chinese yam by using the supercritical fluid CO_2 extraction in this study. Supercritical fluid CO_2 extracted-Chinese yam extracts with anti- hypertensive activity in vitro was verified by applying *in vitro* ACE hypertensive activity inhibition assay. Here is a substantial interest in discovering the supercritical fluid CO_2 extracted-Chinese yam extracts with anti-hypertensive activity *in vivo* in this study.

3. Materials and Methods

3.1. Chemicals and Reagents

All chemicals were ordered from Sigma-Aldrich (St. Louis, MO, USA).

3.2. Source and Storage of Chinese yam (*Dioscorea polystachya*)

Chinese yam was obtained from a Chinese yam planting farm in Taiwan. Theses Chinese yam were washed, dried, and pulverized. Later, the dried powder of Chinese yam was stored at -80°C until used.

3.3. Supercritical Fluid CO_2 Extraction

Supercritical fluid CO_2 extraction was carried out using a supercritical fluid pilot-plant (Metal Industries Research and Development Centre, Taiwan, model SFE-400S-2000R) comprising a 2 L cylinder extraction cell. The temperature and pressure were held constant at 42°C and 350 bars. Extract samples were collected every 30 mins for 5 hours.

3.4. Measurement of Chinese Yam Extract Content by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Spectrophotometer

The content of diosgenin, allantoin, and choline of the Chinese yam extracts was detected by LC-MS/MS. The detection protocol of diosgenin content was used an Waters Atlantis HILIC Silica column (2.1 × 50 mm, 5.0 μm particle size) was used. The mobile phase was set at H_2O : ACN = 10 : 90 (v/v) and flow rate was set at 0.2 mL/min. The detection protocol of choline content was used an ACQUITY UPLC BEH Amide column (2.1 × 50 mm, 1.7 μm particle size) was used. The mobile phase was set at 10 mM $HCOONH_4$: ACN = 60 : 40 (v/v) and flow rate was set at 0.2 mL/min. Mass spectrometry was performed with multiple reaction monitoring (MRM). The ion source adopts electro spray ionization method and analysis was the negative voltage mode. Nebulize was set at 40 psi. Dry gas flow and temperature were set respectively at 8 L/min and 350°C. Capillary voltage was set at 4,000 V. Mass spectrometry parameters of diosgenin, allantoin, and choline are listed in (Table 1). In addition, lecithin analysis method was referred to AOCS Recommended Practice Ca 12-55 Phosphorus. The content of lecithin of the Chinese yam extracts was detected by spectrophotometer (ChromThch CT-2200). The analysis software is M. wave professional 2.0.

Table 1: Mass spectrometry parameters of diosgenin, allantoin, and choline. *Quantitative ions.

Compound name	MRM		Frangmentor	Collision energy
	precursor ion (*m/z*)	product ion (m/z)		
diosgenin	415.3	253.3	92	25
	415.3	271.3*	92	17
allantoin	157.03	97.3*	40	9
	157.03	114.3	40	5
	157.03	140.4	40	5
choline	104.1	60.3*	92	17
	104.1	44.2	92	65

3.5. Animal Care

All animal experiments and animal care were approved by the Institutional Animal Care and Utilization Committee of Agricultural Technology Research Institute, Taiwan. Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) were freely fed a standard laboratory diet with or without the extracts of Chinese yam and the sterile drinking water. All rats kept on a 12-h light/dark cycle at 24-27°C, 60-70% humidity, and an automatic control system.

3.6. Detection of Blood Pressure of Chinese Yam Extracts-administrated SHRs

6-8 weeks old WKY rats (n = 8) and SHRs (n = 24) were raised in GLP Animal Laboratory, ATRI. The Chinese yam extracts were dissolved in sterilized drinking water at one dose of 1,000 mg/kg Body Weight (BW). According to blood-pressure level, 24 SHRs were randomized into 3 groups [the negative control group: 8 rats; the positive control group (20 mg/kg/day nifedipine administration): 8 rats; Chinese yam extracts group [40 mg/kg BW Chinese yam extracts] 8 rats. On the other hand, 8 WKY rats were blank normal control group. The Chinese yam extracts were administrated to SHRs by p.o. using disposable feeding needles (FN-9921, 20G × 1.5"; Kent Scientific, San Diego, CA, USA) once per day for 6 weeks. Twenty mg/kg/day nifedipine was administrated for SHRs as the same method of administration with Chinese yam extracts. The same volume and frequency of sterilized drinking water was administrated to WKY rats by p.o. using disposable feeding needles. The blood pressure values were measured for each group once 2 weeks. The efficacy of Chinese yam extracts on the blood pressure was compared with each group. Following p.o. of Chinese yam extracts or sterilized drinking water in rats, the blood pressure of rats was measured by a tail-cuff method (Model MK-2000ST; Muromachi Kikai, Tokyo, Japan)without warming rats in a chamber maintained at 38°C for 5 min. Five times at a time and take the average for the detection of blood pressure.

3.7. Statistical analysis

Values of blood pressure [the mean blood Pressure (MBP), systolic blood pressure (SBP), and diastolic blood pressure (DBP)], body weight, and liver and kidney function indexes are represented as mean ± standard error of the mean (SEM). Statistical evaluation was performed using Student's *t*-test, one-way analysis of variance (ANOVA), and SAS 8.0 software. Differences between groups were considered statistically significant at $p < 0.05$.

4. Results

4.1. Measurement of Diosgenin, Allantoin, Choline, and Lecithin Content of Chinese Yam Extracts by LC-MS/MS and Spectrophotometer

LC-MS/MS analysis for the simultaneous determination of diosgenin, allantoin, and choline content of Chinese yam extracts were rapid with a high degree of reproducibility. The results showed diosgenin at various concentrations (0, 25, 50, 100, 250, and 500 ng/mL) were used. The standard curve and coefficient of determination (C.V.) r^2 of diosgenin was Y= 4804.254851X + 41475.167359 and r^2 = 0.9806 (Figure 1). An obvious peak of diosgenin **(**100 ng/mL**)** was at 2.859 min (Figure 2). LOQ (limit of quantification) and LOD (limit of detection) for diosgenin was respectively 10.4 ng/mL and 3.1 ng/mL.

The allantoin at various concentrations (0, 25, 50, 100, 250, and 500 ng/mL) were used. The standard curve and coefficient of determination (C.V.) r^2 of allantoin was Y= 21.198324X + 166.503310 and r^2 = 0.9932 (Figure 3). An obvious peak of allantoin **(**100 ng/mL**)** was at 1.409 min (Figure 4). LOQ (limit of quantification) and LOD (limit of detection) for allantoin was respectively 4.87 ng/mL and 1.46 ng/mL.

The choline at various concentrations (0, 25, 50, 100, 250, and 500 ng/mL) were used. The standard curve and coefficient of determination (C.V.) r^2 of choline was Y= 589.748889X – 2572.711234 and r^2 = 0.9944 (Figure 5). An obvious peak of choline **(**50 ppb**)** was at 1.108 min (Figure 6). LOQ (limit of quantification) and LOD (limit of detection) for choline was respectively 4.72 ng/mL and 1.42 ng/mL.

The lecithin analysis method was referred to AOCS Recommended Practice Ca 12-55 Phosphorus. The content of lecithin of the Chinese yam extracts was detected by spectrophotometer (ChromThch CT-2200). The equation of standard curve of lecithin was Y= 0.0884X + 0.0075. r_2 = 0.9987 (Figure 7).

The C.V (%) of precision for diosgenin (20 and 50 ppm) was 8.2% and 14.1%, respectively. The recovery (%) of trueness for diosgenin (20 and 50 ppm) was 90.8% and 102.5%, respectively (Table 2). According to the above results, we concluded that the established LC-MS/MS was able to successfully detect diosgenin.

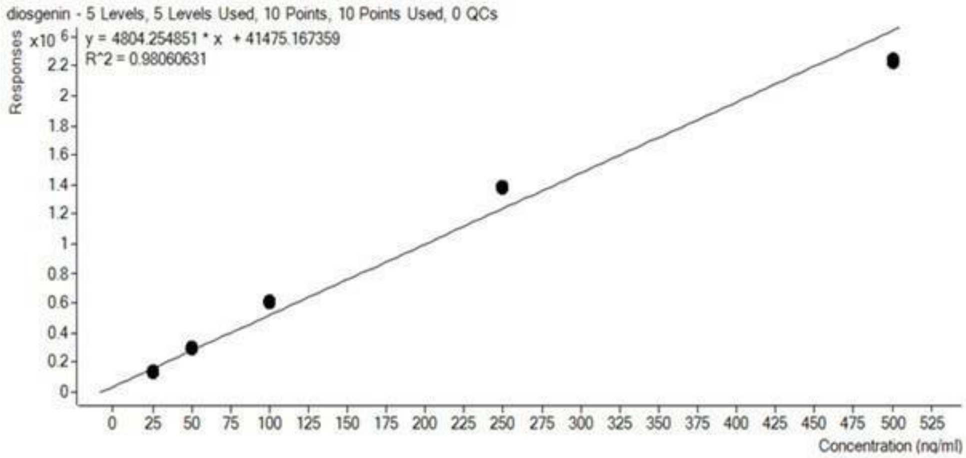

Figure 1: The standard curve of diosgenin.Equation was Y= 4804.254851X + 41475.167359.r^2 = 0.9806.

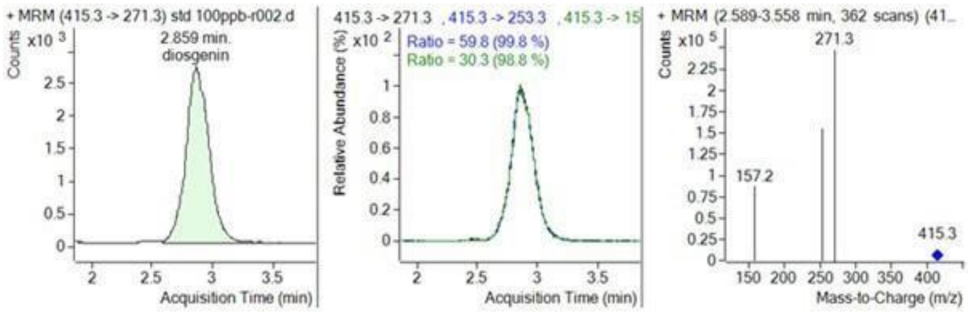

Figure 2: The chromatogram and mass spectrometry parameters of diosgenin.

An obvious peak of diosgenin (100 ng/mL) was at 2.859 min. The ion source adopts electro spray ionization method and analysis was the negative voltage mode.

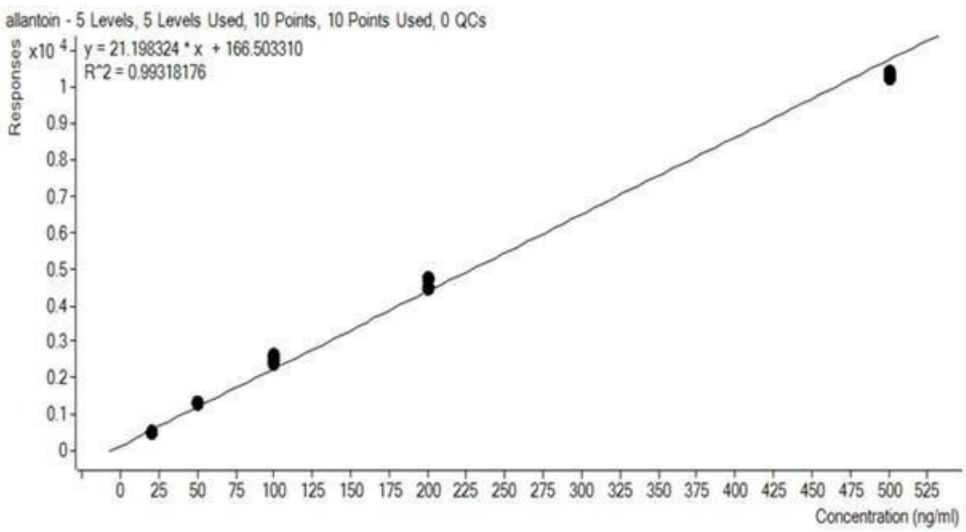

Figure 3: The standard curve of allantoin. Equation was Y= 21.198324X + 166.503310. r_2 = 0.9932.

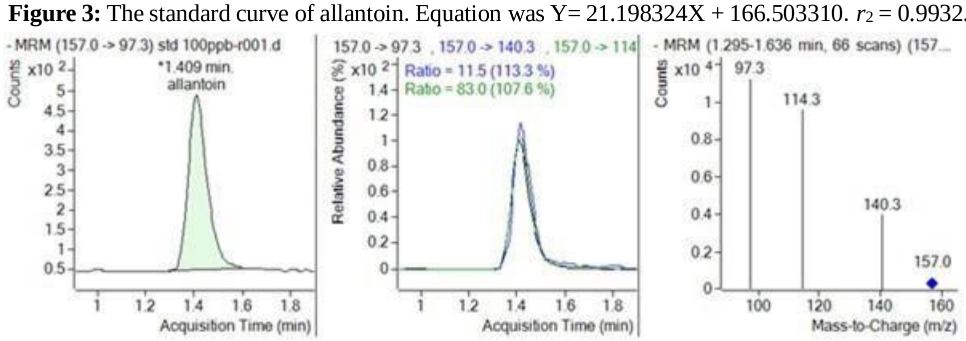

Figure 4: The chromatogram and mass spectrometry parameters of allantoin. An obvious peak of allantoin (100 ng/mL) was at 1.409 min. The ion source adopts electrospray ionization method and analysis was the negative voltage mode.

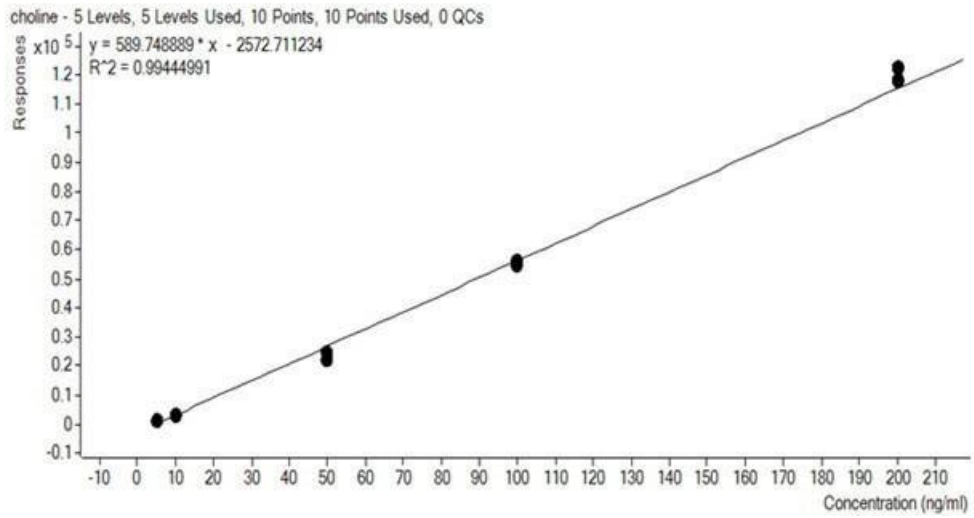

Figure 5: The standard curve of choline. Equation was Y= 589.748889X – 2572.711234. r_2 = 0.9944

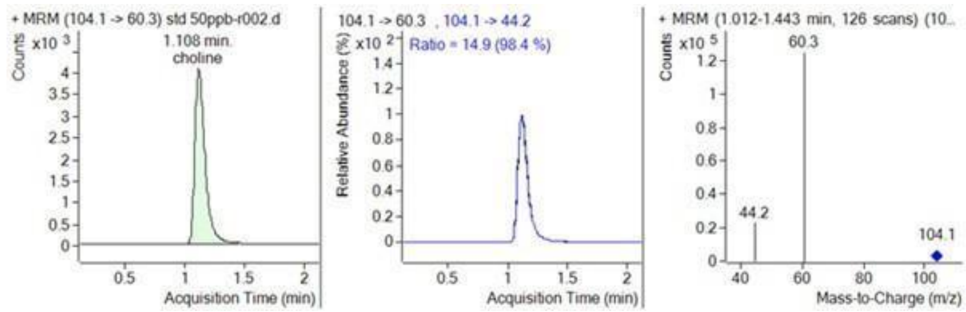

Figure 6: The chromatogram and mass spectrometry parameters of choline.

An obvious peak of choline (50 ng/mL) was at 1.108 min. The ion source adopts electrospray ionization method and analysis was the negative voltage mode.

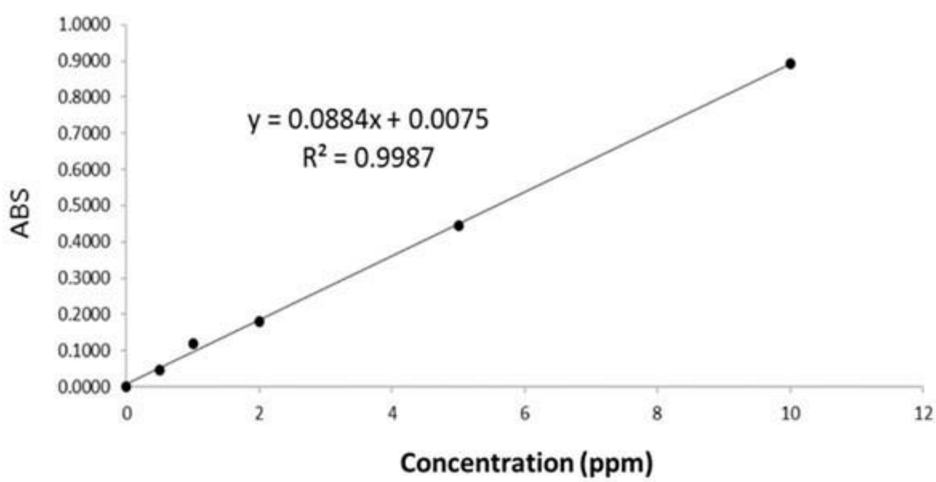

Figure 7: The standard curve of lecithin. Equation was Y= 0.0884X + 0.0075. r_2 = 0.9987.

The C.V. (%) of precision for allantoin (20 and 50 ppm) was 10.9% and 10.1%, respectively. The recovery (%) of trueness for allantoin (20 and 50 ppm) was 108.3% and 96.6%, respectively (Table 3). According to the above results, we concluded that the established LC-MS/MS was able to successfully detect allantoin.

The C.V. (%) of precision for choline (10 and 20 ppb) was 4.0% and 0.7%, respectively. The recovery (%) of trueness for choline (10 and 20 ppb) was 116.2% and 111.6%, respectively (Table 4). According to the above results, we concluded that the established LC-MS/MS was able to successfully detect choline.

Additionally, one Chinese yam extracts was provided to detect the concentrations of diosgenin, allantoin, choline, and lecithin. These results of diosgenin, allantoin, choline, and lecithin concentrations in one supercritical fluid CO_2 extract were respectively 0.13 μg/g, 5743.42 μg/g, 812.61 μg/g, and 3.8 g/100 g (Table 5).

Table 2: Recovery and C.V. of diosgenin (20 and 50 ppm).

Compound	Spike level (ppm)	Recovery (%)	C.V. (%)
diosgenin	20	90.8	8.2
	50	102.5	14.1

Table 3: Recovery and C.V. of allantoin (20 and 50 ppm).

Compound	Spike level (ppm)	Recovery (%)	C.V. (%)
allantoin	20	108.3	10.9
	50	96.6	10.1

Table 4: Recovery and C.V. of choline (10 and 20 ppb).

Compound	Spike level (ppb)	Recovery (%)	C.V. (%)
choline	10	116.2	4.0
	20	111.6	0.7

Table 5: Detection of diosgenin, allantoin, choline, and lecithin content of Chinese yam extracts.

Functional components	Concentration
diosgenin	0.13 μg/g
allantoin	5743.42 μg/g
choline	812.61 μg/g
lecithin	3.8 g/100g

4.2. Effect of Chinese Yam Extracts on Body weight (BW), Blood Hypertension Regulation, and Liver and Kidney Functions *in Vivo*

The once per day-p.o. experiments with Chinese yam extracts in 6-8 weeks old SHRs. Twenty-four SHRs were randomized into 3 groups: the negative control group, the positive control group (nifedipine administration),and Chinese yam extracts group [40 mg/kg Chinese yam extracts]. Eight WKY rats were blank normal control group. Chinese yam extracts were administrated by p.o. through gavage per day for 6 weeks. The BW values were measured for each group once a week and the blood pressure values were measured for each group once 2 weeks. The BW of all rats were increase continuously and were showed non-significantly different ($p > 0.05$) between 4 groups each other (Table 6).The blood pressure of SHRs before and after experiment in three groups were respectively 135.50 ± 4.51 mmHg; 169.75 ± 9.84 mmHg (the negative control group), 139.75 ± 10.75 mmHg; 102.17 ± 4.39 mmHg (the positive control group), and 152.53 ± 6.69 mmHg; 151.07 ± 8.85 mmHg (*Dioscorea polystachya* extract). The efficacy of anti-hypertension of nifedipine is significantly higher than those of Chinese yam extracts ($p < 0.001$). The mean blood pressure in Dioscorea polystachya extract group was lower than negative control group, but not presents significant difference (Figure 8). Chinese yam extracts have a potential ability to regulate blood pressure. Moreover, 4 indexes (GOT, GPT, BUN, and CRE) of the liver and kidney functions of Chinese yam extracts-administrated SHRs were located at the normal levels (Table 7).

5. Discussion

Tubers of Chinese yam are commonly used as cooking vegetables, but their starchy flour is also used for baking and preparing noodles [11-13]. The tubers of Chinese yam not only provide nutrients, but also contain various bioactive ingredients. Therefore, Chinese yam is also used and applied in the traditional Chinese medicine and modern herbal remedies [14].

According the references, Chinese yam extracts have been verified that they can reduce serum levels of low-density lipoprotein cholesterol, inhibition of fat absorption, regulation of hypertension, attenuation of obesity, and reduce the incidence of diabetes [15-17].

Table 6: Change of body weight in WKY rats and SHRs during Chinese yam extracts-oral administration period. period. Data were presented as mean ± SEM.

Week	WKY rats	SHRs		
	Normal control	Negative control	Positive control	*Dioscorea polystachya extract*
0	224.34 ± 1.85 g	237.28 ± 5.94 g	245.03 ± 4.52 g	249.94 ± 3.27 g
2	256.82 ± 2.07 g	261.83 ± 10.49 g	269.18 ± 9.30 g	265.06 ± 5.56 g
4	287.32 ± 2.87 g	279.90 ± 12.52 g	288.18 ± 10.66 g	281.36 ± 5.15 g
6	317.20 ± 4.17 g	300.55 ± 16.32 g	312.43 ± 14.72 g	300.96 ± 6.68 g

Table 7: Effects of Chinese yam extracts on liver and kidney function of WKY rats and SHRs. Data were presented as mean ± SEM. Abbreviation: Wistar Kyoto (WKY); spontaneously hypertensive rats (SHRs); standard error of the mean (SEM); Glutamic-oxaloacetic transaminase (GOT); glutamic-pyruvic transaminase (GPT); blood urea nitrogen (BUN); creatinine (CRE).

Beginning	WKY rats	SHRs		
	Normal control	Negative control	Positive control	*Dioscorea polystachya extract*
GOT (U/L)	101.60 ± 10.92	91.50 ± 7.24	80.00 ± 1.47	78.10 ± 2.18
GPT (U/L)	46.40 ± 2.26	33.50 ± 0.87	36.75 ± 1.44	32.40 ± 1.50
BUN (mg/dL)	20.12 ± 0.87	24.95 ± 1.26	27.10 ± 2.29	25.42 ± 1.09
CRE (mg/dL)	0.20 ± 0.02	0.18 ± 0.03	0.18 ± 0.03	0.14 ± 0.02
End				
GOT (U/L)	79.00 ± 5.54	135.75 ± 17.82	91.50 ± 1.32	81.40± 2.18
GPT (U/L)	37.00 ± 1.67	51.25 ± 4.75	34.25 ± 1.31	38.00 ± 1.52
BUN (mg/dL)	17.76 ± 0.66	21.55 ± 0.70	21.25 ± 1.46	18.04 ± 0.71
CRE (mg/dL)	0.24 ± 0.02	0.28 ± 0.02	0.20 ± 0.00	0.24 ± 0.02

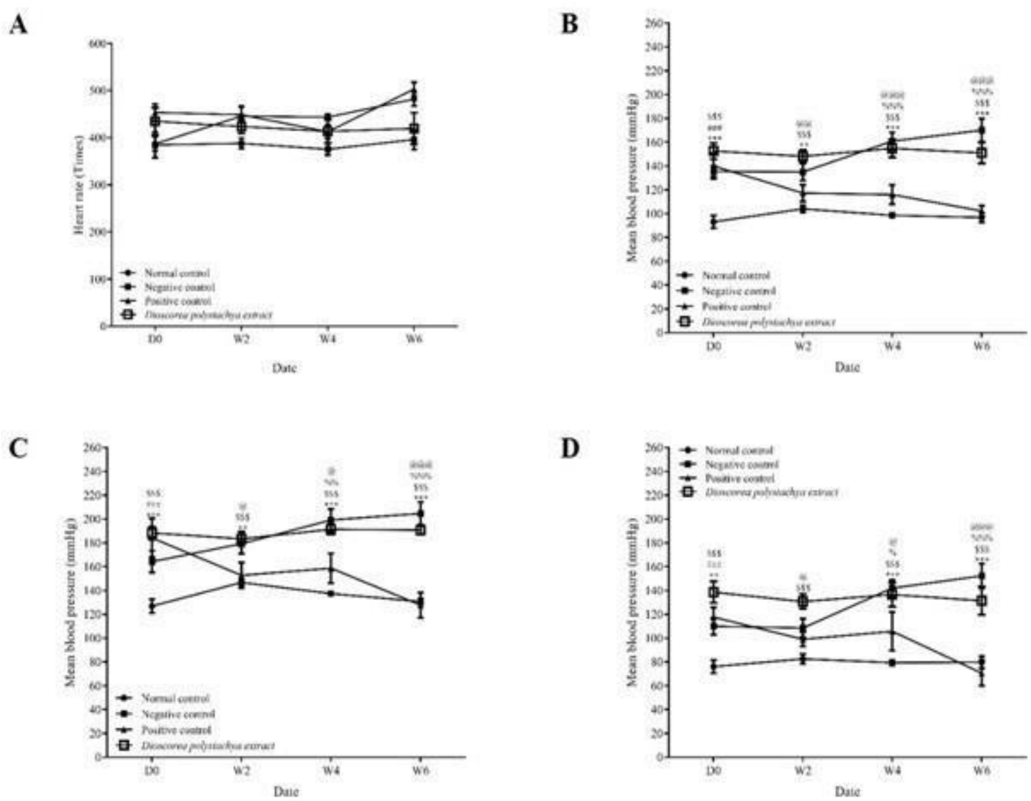

Figure 8: Efficacy of the Chinese yam extracts on the anti-blood hypertension *in vivo*. Twenty-four SHRs were randomized into 3 groups [the negative control group: 8 rats; the positive control group (20 mg/kg/day nifedipine): 8 rats; Chinese yam extract group (*Dioscorea polystachya* extract) (40 mg/kg BW): 8 rats]. Eight WKY rats were blank normal control group. (A) Heart rates; (B) MBP; (C) SBP; (D) DBP. Data were presented as mean ± SEM. *: Normal control vs. Negative control; #: Normal control vs. Positive control; $: Normal control vs. *Dioscorea polystachya* extract; %: Negative control vs. Positive control; &: Negative control vs. *Dioscorea polystachya* extract; @: Positive control vs. *Dioscorea polystachya* extract. $^{@/\%}p < 0.05$; $^{**}p < 0.01$; $^{***/\$\$\$/\%\%\%/@@@}p < 0.001$. Abbreviation: Wistar Kyoto (WKY); spontaneously hypertensive rats (SHR); standard error of the mean (SEM); mean blood pressure (MBP); systolic blood pressure (SBP); diastolic blood pressure (DBP).

In addition, Chinese yam is rich in antioxidants and have a positive effect on the immune system [18]. Anti-tumor and periodic effects have also been reported [19-20].

The ingredients of Chinese yam with pharmacological activity are mainly polysaccharides (such as resistant starches), as well as sapogenins (especially diosgenin) and the storage protein dioscorin, allantoin, choline, and lecithin [9]. Chinese yam is a potential source of nutritional and bio-functional food ingredients. Previously, we have demonstrated that supercritical fluid CO_2 extracted-*Citrus* depressa Hayata extracts possessed the hypertension regulation ability [2]. In this study, supercritical fluid CO_2 extracted-Chinese yam extracts were orally administrated to SHRs by gavage for 6 weeks and evaluated the effects of supercritical fluid CO_2 extracted-Chinese yam extracts in regulating hypertension. *In vivo* results were showed that the body weight in each group was not significantly different with or without oral administration with supercritical fluid CO_2 extracted-Chinese yam extracts. After 6 weeks administration of supercritical fluid CO_2 extracted-Chinese yam extracts in SHRs, the MBP, SBP, and DBP were decreased. Taken these results together, supercritical fluid CO_2 extracted-Chinese yam extracts have a potential ability to regulate hypertension. In addition, the supercritical fluid CO_2 extracted-Chinese yam extracts is safe for the experimental rat consumption according to the liver and kidney functional indexes (GOT, GPT, BUN, and CRE).

6. Conclusion

Our study mainly focused on the anti-hypertension efficacy of the supercritical fluid CO_2 extracted-Chinese Yam (*Dioscorea polystachya*) extracts. The present study indicated Chinese yam (*Dioscorea polystachya*) extracts had the regulation of blood pressure and no side-effects were found *in vivo*. Our data might contribute to further research into food derived anti-hypertensive compounds, meanwhile it also provides some reference for the clinical drug use of Chinese yam in the traditional Chinese medicine.

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