Research Article

The effectiveness of ethanol extract of purple sweet potato var. *Ayamurasaki* as a natural antihypertensive mitigator in deoxycorticosterone acetate-salt hypertensive rats

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ABSTRACT

Backgorund: There is an increasing amount of evidence that oxidative stress related to hypertension can damage the function of diverse structures such as the aorta and kidney. It is a well-established fact that chlorogenic acid and anthocyanin found in purple sweet potato generate bioactive compound with antihypertensive and antioxidative activities. **Purpose:** The present study sought to investigate blood pressure-lowering effect and antioxidative activity of ethanol extract of purple sweet potato (EP) in deoxycorticosterone acetate/sodium chloride (DOCA–salt)-induced hypertensive rats (Rattus norvegicus). **Methods:** Rats were orally administrated 95% ethanol extract of purple sweet potato (var. Ayamurasaki) in a daily dose of 100 and 200 mg/kg body weight for 4 weeks. Systole blood pressure (SBP) and renal and aorta total malondialdehyde (MDA) were assessed. **Results:** Mean SBP was lowered in the hypertensive rats following EP therapy from their values at the time of administration. Renal and aorta injury was observed in the DOCA–salt hypertensive rats compared to normotensive group rats; renal and aorta MDA significantly increased ($P < 0.05$). In contrast, treatment of DOCA–salt in hypertensive rats with a different dose of EP significantly reduced the total renal and aorta MDA. **Conclusion:** This is the first report that demonstrates the lowering of blood pressure and antioxidative effects of an ethanol extract of purple sweet potato, containing chlorogenic acid, in a DOCA–salt model of hypertension.

KEY WORDS: Antioxidative, Blood pressure, Chlorogenic acid

INTRODUCTION

Hypertension is a major cause of mortality associated with cardiovascular disease, cerebrovascular disease, and renal disease. It has a prevalence of 26.4% in the adult population, totaling nearly 1 billion individuals, and it has been estimated that it will increase up to 29% (1.5 billion) by the year 2025.[1] The renin-angiotensin-aldosterone system (RAAS) is a hormonal cascade that has various functions in the pathogenesis of cardiovascular diseases. Angiotensin-II, a potent vasoconstrictor, is the primary active product of the RAAS that plays a central role in the development of hypertension.[2] Hypertension also has been associated with stress oxidation, which results from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system.[3] In this case, ROS production by NADPH oxidase is increased, causing vascular disease and dysfunction. ROS production in other organs, particularly the kidney, likely contributes to blood pressure regulation.[4] The deoxycorticosterone acetate (DOCA)–salt-induced rat model is an endocrine hypertension model that progresses quickly to severe hypertension and oxidative stress,[5] allowing an understanding of progression of the disease and testing of potential therapies, such as, here, the potential use of ethanol extract of purple sweet potato (EP), containing chlorogenic acid as the therapeutic agent.

Purple sweet potato is known to have several advantages over other sweet potatoes in terms of potential hypertension reduction. It contains anthocyanins, dioscorin protein, and chlorogenic acid, which has been noted to have antihypertensive and antioxidative activity. Chlorogenic acid can inhibit the angiotensin-converting enzyme

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(ACE), which has an important role in converting angiotensin-I to angiotensin-II by blocking the active site of the enzyme.[6] Angiotensin-II is both a potent vasoconstrictor and stimulator for the synthesis and release of aldosterone, which subsequently increases blood pressure by promoting sodium retention in the distal tubules.[7] Given the above considerations, the inhibition of ACE could be useful in the treatment of hypertension.[8] Antioxidative activity showed the activity of scavengers against superoxide anion, radicalhydroxyland2,2-diphenyl-1-picrylhydrazyl.[9]

Hence, the administration of antioxidants is considered a useful therapeutic approach in the treatment of high blood pressure. To understand dose-dependent effects of EP-derived chlorogenic acid treatment on systole blood pressure (SBP) and malondialdehyde (MDA) level, we used DOCA–salt-induced hypertension Wistar rat (*Rattus norvegicus*) strains as an animal model. The current study demonstrated a decrease in SBP, reduction of MDA level in the aorta and kidney, and also repairing of kidney and aorta damage after a single oral administration of purple sweet potato ethanol extract in DOCA–salt-induced hypertension rats.

MATERIALS AND METHODS

Chemicals

Purple sweet potatoes var. *Ayamurasaki*; 95% ethanol; DOCA (Sigma, Pcode 1001376001, USA); NaCl; and corn oil (Sigma, Pcode 1000925370 C8726-500 ml) were used.

Animal Model

All procedures were carried out in accordance with conventional guidelines for experimentation with animals. Twelve-week-old male Wistar rat (*R. norvegicus*) strains were used. The rats were divided into five groups, i.e., normotensive (NTN) (A); hypertensive (HTN) (B), HTN + ethanol extract (EP) at one-half standard dose (100 mg/kg) b/w (C); and HTN + EP standard dose of 200 mg/kg b/w (D) and were housed in groups of five per cage, as a number for replication, in a regulated environment with a 12 h light/dark cycle. HTN rats were prepared by induction of DOCA, twice a week for 5 weeks (10 injections). For administration, DOCA was dissolved in 0.5 ml corn oil.[10,11] DOCA was injected subcutaneously in the cervical spine with the first five doses being 20 mg/kg and the last five doses 10 mg/kg. Rats were administered 1% NaCl through their drinking water. Rats in Groups C and D received, by oral administration, using a cannula, a daily dose of EP at either 100 or 200 mg/kg body weight, respectively, dissolved in reverse osmosis water, for 4 consecutive weeks. The control group received a normal diet.

Preparation of EP

Purple sweet potatoes were sorted and weighed then washed with clean water. After that, the sweet potatoes were sliced into small pieces and blended with 95% ethanol at a ratio of 1:8 (v/v) potato: ethanol for the 30 s. The suspension was then screened and macerated for 2 × 12 h. After that, the solution was filtered again by vacuum screening and Whatman paper at 40 until getting the filtrate for evaporating and their residue extracting again until 4 times, which was used for the further experiments.

Identification of Chlorogenic Acid from Purple Sweet Potato Ethanol Extract by Liquid Chromatography–Mass Spectrometry (LC–MS)

Purple sweet potato flour dissolved in 5% acetonitrile and 0.1% formic acid in deionized water was used for LC–MS/MS analysis. LC–MS/MS analysis used the LCQ Deka XP system MAX Thermo with electrospray ionization (Thermo Scientific Inc., USA) with a C18 BioBasic column, diameter 150 mm × 2.1 mm, particle size 5 µm. LC–MS running conditions were air flow speed 50 arb, sprayer tension of 4 kV, caliper tension of 20 V, and caliper temperature 300°C. MS scans were at an interval m/z 100–m/z 1600 with a flow rate of 200 µl/min. Isolation of chlorogenic acid was by a linear gradient with order size 5% solvent B until 70% solvent B (0.1% formic acids in acetonitrile) during a 90-min run. Mass spectra were read with Thermo Xcalibur™ (Thermo Scientific, USA program). Chromatogram LC–MS/MS from Thermo Xcalibur™ became MGF files with Mascot Distiller v2.3.2.0 (Matrix Science, UK).

Measurement of SBP

SBP was measured in awake rats using the tail-cuff method with a photoelectric sensor (blood pressure analyzer, IITC, Model 179, Woodland Hills, USA) as previously described.[12] SBP was measured weekly before DOCA induction, post-induction, and also up to 4 weeks post-administration of EP. SBP was recorded as the average of at least three readings, taken in a quiescent state.

MDA Levels

Levels of MDA, which is a marker of lipid peroxidation, were measured using the TBA reaction method in kidney and aorta tissue homogenates from treated and untreated DOCA–salt rats and NTN rats using a prior adopted method.[13]

Statistical Analysis

The results of SBP and MDA measurements were expressed as means ± standard deviation. Differences between trial groups were statistically analyzed using analysis of variance followed by the *post hoc* Tukey test for determining significant differences at $P < 0.05$.

RESULTS

Identification of chlorogenic acid from purple sweet potato ethanol extract by LC–MS shown in Figure 1.

Effect of EP on SBP

Induction of DOCA–salt 2 times weekly for 5 weeks at the dose of 20 mg/kg bb (×5 injections) followed by 10 mg/kg bb (×5 injections) produced a gradual elevation of SBP [Figure 2].

The mean SBP of DOCA–salt-induced rats (HTN) was significantly higher (201.25 ± 2.06 mmHg) in comparison to NTN rats in basal conditions (108.00 ± 2.94). In contrast, the SBP of HTN rats treated with EP significantly decreased. The final SBPs at the end of the experiment (week 10) were 144.00 ± 2.45 mmHg and 152.75 ± 2.36 mmHg, respectively.

Effect of EP on MDA Levels in the Kidney and Abdominal Aorta

The serum levels of MDA as the markers of oxidative stress in kidney and abdominal aorta of the NTN, the HTN, and EP-treated HTN rats are shown in Tables 1 and 2. Induction of NTN rats with DOCA–salt for 5 weeks resulted in an increase of MDA level. The MDA level of DOCA–salt HTN rats was significantly differed ($P < 0.05$) compared to NTN rats.

DISCUSSION

Sweet potatoes contain phenolic esters that act as antioxidants, such as chlorogenic acid, chlorogenic

Table 1: Effect of EP on the levels of MDA in kidney of deoxycorticosterone acetate–salt hypertensive rats (means±SD) (*n*=4)

Groups	MDA level (mean±SD) µg/ml	Increase in MDA level (%) compared to normotensive	Reduction in MDA level (%) compared to hypertensive
Normotensive	0.332±0.066[a]	-	-
Hypertensive (HTN)	0.694±0.073[c]	109.03	-
HTN+EP 100	0.462±0.024[b]	-	33.42
HTN+EP 200	0.408±0.049[ab]	-	41.21

*Values not sharing a common superscript differ significantly at $P<0.05$. MDA: Malondialdehyde

Table 2: Effect of EP on the levels of MDA in abdominal aorta of DOCA–salt HTN rats (means±SD) (*n*=4)

Groups	MDA level (mean±SD) µg/ml	Increase in MDA level (%) compared to normotensive	Reduction in MDA level (%) compared to hypertensive
Normotensive	0.318±0.087[a]	-	-
Hypertensive (HTN)	0.612±0.097[b]	92.45	-
HTN+EP 100	0.439±0.081[a]	-	28.26
HTN+EP 200	0.428±0.046[a]	-	30.06

*Values not sharing a common superscript differ significantly at $P<0.05$. MDA: Malondialdehyde

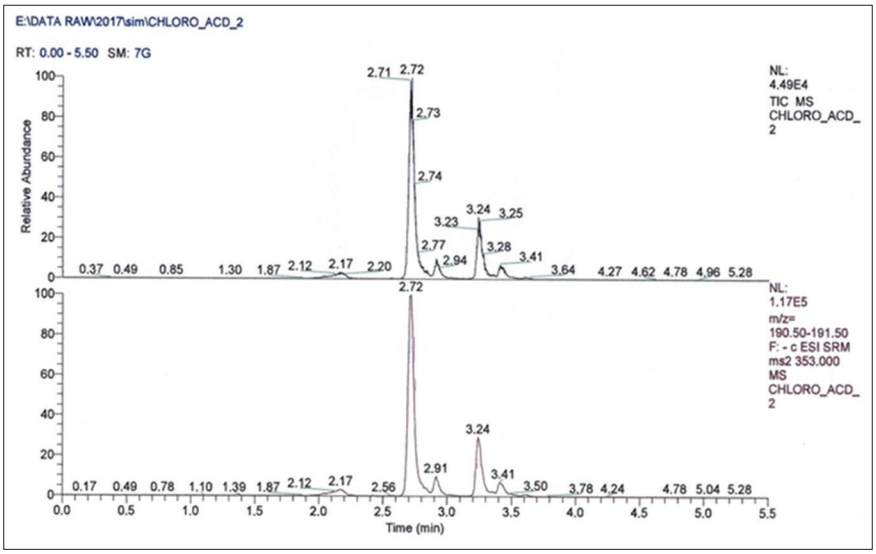

Figure 1: Liquid chromatography–mass spectrometry (LC–MS)/MS chromatogram of chlorogenic acid from purple sweet potato var. *Ayamurasaki* ethanol extract. The result of LC–MS showed that chlorogenic acid in the ethanol extract had a retention time of 2.72 min (P)

Table 3. Effect of EP in Systole and Diastole Blood Pressure of DOCA salt rats (means±SD) (n=4)

Groups	Systole BP (mmHg)	Diastole BP (mmHg)
Normotensive	111± 3,49a	89,2± 0,84a
Hypertensive	177± 2,68c	118,2± 1,10c
HTN +EP 100	125± 0,84b	110,0± 1,41b
HTN +EP 200	114± 4,03a	89,2± 1,10a

*Values not sharing a common superscript differ significantly at $P<0.05$.
BP = Blood Pressure

acid, and affect acid. The phenolic component in sweet potato blocks the presence of free radicals (antioxidant) and acts as antihypertensive agents. In 100 g of the tuber, there were 11.2 mg chlorogenic acid and 7.1 mg isochlorogenic acid with an overall phenolic content of 18.6 mg.[6]

Structurally, chlorogenic acid is a caffeic acid ester having a 3-hydroxyl unit with formula $C_{16}H_{18}O_9$. The phenol content of purple sweet potatoes is 4.9–6.7 times higher than yellow and white sweet potatoes.[6] Purple sweet potatoes have the highest phenol content followed by orange sweet potatoes and then white sweet potatoes.[14] The form of phenol esters composing most of the tubers is chlorogenic acid.

Effect of EP on SBP and DBP

Induction of DOCA–salt 2 times weekly for 5 weeks at the dose of 20 mg/kg bb (×5 injections) followed by 10 mg/kg bb (×5 injections) produced a gradual elevation of SBP and DBP [Table 3]. The mean SBP of DOCA–salt-induced rats (HTN) was significantly higher (177 ± 2.68 mmHg) in comparison to NTN rats in basal conditions (111 ± 3.49). In contrast, the SBP of HTN rats treated with EP significantly decreased. The final SBPs at the end of the experiment (week 10) were 125.00 ± 0.84 mmHg and 114 ± 4.03 mmHg, respectively. The DBP of HTN rats treated with EP significantly also decreased. The final DBPs at the end of the experiment (week 10) were 110 ± 1.41 mmHg and 89.2 ± 1.10 mmHg.[15]

Treatment of HTN rats with EP at the dose of 200 mg/kg bw gave a better antihypertensive effect. In this study, EP showed blood pressure-lowering effect and caused major changes of the SBP in the treated DOCA–salt HTN rats of more than 50 mmHg. In contrast to the present study, the blood pressure-lowering effect of EP was lower (15 mmHg).

There was a considerable increase in MDA in kidney tissue, up to 109.03%, and 92.45% in the abdominal aorta of DOCA–salt HTN rats. These findings were evidence of renal injury and aorta damage related to oxidative stress, thus indicating an increase in the oxidative stress levels in the HTN rats as compared to those in the NTN group. Elevation of blood pressure in the DOCA–salt model may activate oxidative stress through an unregulated NADPH oxidase.[16]

Oral administration of 100 and 200 mg/kg bw of EP for a period of 4 consecutive weeks to the HTN rats significantly reduced MDA level ($P < 0.05$). However, there was not a significantly different effect of the dose of EP to the renal and aorta MDA level of the treated HTN rats. EP at a dose of 200 mg/kg bw gave a better effect in reducing renal MDA levels (41.21%) as compared to the dose of 100 mg/kg bw (33.42%). Several studies have reported the antioxidative activity of chlorogenic acid[17-20]; to the best of our knowledge, this is the first study reporting chlorogenic acid derived from an ethanol extract of purple sweet potatoes which has an antioxidative activity that may reduce the effect of oxidative stress in DOCA–salt HTN rats. This study also demonstrated that bringing the blood pressure to the NTN state using EP, which contains antihypertensive compound like chlorogenic acid not only decreased the blood pressure but also attenuated organ damage.

CONCLUSION

Assessment of the antihypertensive properties of purple sweet potato ethanol extract-derived chlorogenic acid in the present study showed a significant effect in reducing SBP and attenuating renal and abdominal aorta damages in a DOCA–salt HTN rat model. These findings suggest that purple sweet potato ethanol extract-derived chlorogenic acid might be considered as a potential antihypertensive agent that is resistant to digestive proteases. However, further investigation is needed to evaluate the bioavailability of sweet potato-derived chlorogenic acid after digestion, which can be a significant factor impacting the preparation and efficacy of nutraceutical food components.

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