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Malonaldehyde Level of Administration Ethanol Extract of Purple Sweet Potato Var. Ayamurasaki in Doca-Salt Hypertensive Rats

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Abstract

There is an increasing amount of evidence that oxidative stress related to hypertension can damage the function of diverse structures such as aorta. It is a well-established fact that chlorogenic acid and anthocyanine found in purple sweet potato generates bioactive compound with antihypertensive and antioxidant activities. The present study sought to investigate antioxidant activity of extract ethanol of purple sweet potato (EP) in *deoxycorticosterone acetate* (DOCA–salt)–induced hypertensive rats (*Rattus norvegicus*). The rats were orally administrated a 95% ethanol extract of purple sweet potato (var. *Ayamurasaki*) (EP) in a daily dose of 200 and 400 mg/kg body weight for 4 weeks. Aorta total malondialdehyde (MDA) and histopathology of aorta abdominal were examined. Aorta injury was observed in DOCA-salt hypertensive group rats compared to normotensive group rats, as aorta MDA significantly increased ($P<0.05$). In contrast, treatment of DOCA-salt hypertensive rats with different dose of EP significantly reduced the total aorta MDA, as well as repair kidney damage, suppressed smooth muscle cell proliferation and lessen aorta wall thickening compared to controls. This is the first report that demonstrated blood pressure lowering and antioxidative effects of an ethanol extract of purple sweet potato, containing chlorogenic acid, in a DOCA–salt model of hypertension.

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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 one billion individuals, and it has been estimated that it will increase up to 29% (1.5 billion) by the year 2025 (Rubattu *et al.*, 2015). 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 (Atlas, 2007). 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 (Vaziri, 2008). 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 (Harrison *et al.*, 2007). The DOCA-salt–induced rat model is an endocrine hypertension model that progresses quickly to severe hypertension and oxidative stress (Dornas and Silva, 2011), 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, 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. Chlorogenic acid can inhibit the angiotensin converting enzyme (ACE), which has an important role in converting angiotensin-I to angiotensin-II by blocking the active site of the enzyme (Yashimoto *et al*., 1999). 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 (Ferrario, 2010). Given the above considerations, the inhibition of ACE could be useful in the treatment of hypertension (Ramos-Nino and Blumen, 2009).

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

Materials and Methods

This research used purple sweet potatoes var. *Ayamurasaki*; 95% ethanol; deoxycorticosterone acetate (DOCA) (Sigma, Pcode 1001376001, USA); NaCl; corn oil (Sigma, Pcode 1000925370 C8726-500 ml).

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 (200 mg/kg) b/w (C); HTN + EP standard dose of 400 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. Hypertensive rats were prepared by induction of deoxycorticosterone acetate (DOCA), twice a week for five weeks (10 injections). For administration, DOCA was dissolved in 0.5 ml corn oil (Badyal et al., 2003; Khorsid et al., 2012). 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 via their drinking water. Rats in groups C and D,E received, by oral administration, using a canula, a daily dose of EP at either 200 or 400 mg/kg body weight, respectively, dissolved in reverse osmosis water, for 4 consecutive weeks. The control group received a normal diet.

Preparation of purple sweet potato ethanol extract (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 30s. The suspension was then screened and macerated for 2 x 12 hours. After that, the solution was filtered again by vacuum screening and Whatman paper[@] until getting the filtrate for evaporating and their residue extracting again until 4 times, which was used for the further experiments.

Malondialdehyde (MDA) level

Malondialdehyde (MDA) level which is a marker of lipid peroxidation was measured using the TBA reaction method in kidney and aorta tissue homogenates from treated and untreated DOCA-salt rats and normotensive rats using a prior adopted method.

Histopathology of kidney and abdominal aorta

All rats were deeply anesthetized with Chloroform 10%. Abdominal aorta were fixed in cold 10% buffered formalin, the tissues were then transversally trimmed and submitted to a routine process for paraffin embedding. The abdominal aorta sections were prepared, deparaffinised, and stained with haematoxylin and eosin (H&E) for histology analysis.

Statistical Analysis

The results of MDA were expressed as means ± standard deviation (SD). Differences between trial groups were statistically analysed using analysis of variance (ANOVA), followed by the post hoc Tukey test for determining significant difference at $p < 0.05$.

Table 1. Effect of extract ethanol purple sweet potato (EP) on the levels of Malondialdehyde (MDA) in abdominal aorta of DOCA-salt hypertensive rats (mean±SD) (n=4)

Groups	MDA Level(μg/ml)	Increase in MDA level (%)	Reduce in MDA level (%)
Normotensive	2.32±0.28a	-	-
Hypertensive	6.02±0.19b	159.48	-
HTN + EP 200	3.69±0.11a	-	38.71
HTN + EP 400	2.60±0,18a	-	56.81

Note: Values not sharing a common superscript differ significantly at P<0.05. *EP: Extract ethanol of Purple Sweet Potato; CA: Chlorogenic Acid

Results and Discussion

Effect of extract ethanol of purple sweet potato

The serum levels of MDA as the markers of oxidative stress in abdominal aorta of the normotensive (NTN), the hypertensive (HTN) and the casein - treated hypertensive rats were shown in Table 1. Induction of normotensive rats with DOCA-salt for 4 weeks resulted in increased of MDA level. The MDA level of DOCA-salt hypertensive rats was significantly differ (P<0.05) compared to normotensive rats. There was considerably increased of MDA up to 159.4 % in abdominal aorta of DOCA- salt hypertensive rats. These finding was evidence of aorta damages related to stress oxidative, thus indicating an increase in the oxidative stress levels

in the hypertensive rats as compared to those in the normotensive group. Elevation of blood pressure in the DOCA-salt model may activate oxidative stress through an unregulated NADPH oxidase. Oral administration of 200 and 400 mg/kg bw of EP for a period of four consecutive weeks to the hypertensive rats significantly reduced MDA level ($p<0.05$). However, there was significant different effect of the dose of EP to the aorta MDA level of the treated hypertensive rats. Eventhough, EP at the dose of 400 mg/kg bw gave better effect in reducing aorta MDA level (56.81%) as compared to the dose of 200 mg/kg bw (38.71%). Several studies have reported the antioxidative activity of food peptides. To our knowledge, this is the first study reporting the extract ethanol of purple sweet potato (EP) with antioxidative activity that may reduce the effect of oxidative stress in DOCA-salt hypertensive rats. This study also demonstrated that bringing of the blood pressure to the normotensive state using EP containing bioactive compound not only decreased the blood pressure but that also attenuated organ damages.

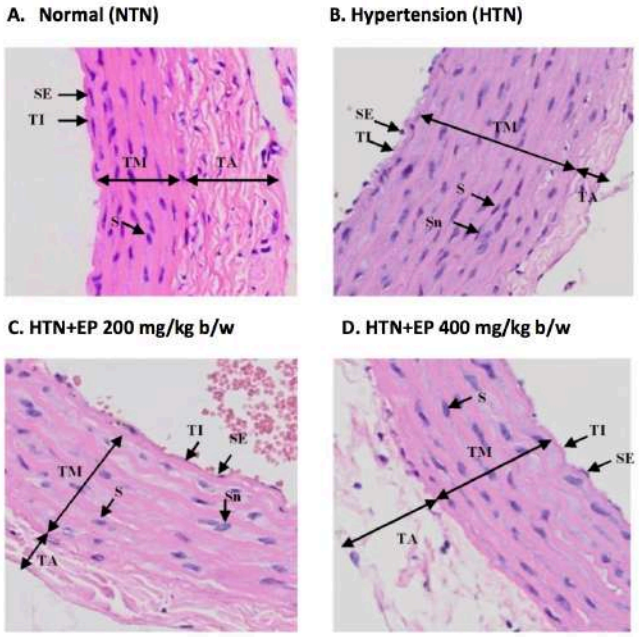

Figure 1.Histopathological changes in the aorta (HE, 40x). Normotensive-rat Aorta (A); DOCA-salt hypertensive (HTN) aorta (B); HTN + EP (200 mg/kg bw) treated aorta(C); HTN + EP (400 mg/kg bw) treated aorta(D); (EC) Endotel cell; (SC) Smooth Muscle cell; (SCn) Necrotic Smooth Muscle cell; (TI) *Tunica Intima;* (TM) *Tunica Media;* (TA) *Tunica Adventitia*. EP=ethanol extract purple sweet potato; CA =chlorogenic acid

Histopathology of aorta abdominal

Histological analysis of the aorta abdominal in normotensive rats was showing normal glomeruli and tubules, and histopathological changes was observed in the architecture of aorta abdominal of all DOCA-salt hypertensive group (Figure 1).

Hypertension is associated with structural and functional alterations of blood vessels. Figure revealed that the aorta abnominal of normotensive rats (A) showed no histological changes, while in the hypertensive rats (B), there were thickening of tunica media (TM) and alteration of tunica intima (TI), increased numbers of smooth muscle cells (S) as well as the evidence of necrotic smooth muscle cells (Sa). Disruption of tunica intima, marked hypertrophy and thickening with proliferation of myocytes in tunica media was reported in histological studies with rats submitted to DOCA-salt hypertension. The tunica media thickness (TM) and amount of necrotic smooth muscle cells (Sa) were substantially changed by administration of EP for 4 weeks, in the present study (Fig 1C and 1D). These finding suggested a significant effect of this treatment on the development of hypertension. Although many evidence clearly indicated the antioxidative activity of EP, few study had evaluated the antioxidant effects exerted by extract ethanol of purple sweet potato (EP) in DOCA-salt hypertensive model.

Conclusion

Assessment of the antihypertension and antioxidative properties of extract ethanol of purple sweet potato (EP) in the present study showed a significant effect in attenuating abdominal aorta damages in DOCA-salt hypertensive model. These findings have suggested that the extract ethanol of purple sweet potato (EP) with high bioactive content like chlorogenic acid and anthocyanin might be considered as potential antihypertension and antioxidants that resistant to digestive proteases. However, further investigation is needed to evaluate bioavailability and efficacy of an purple sweet potato (EP) after GI digestion, which was significant for the preparation of nutraceutical food component.

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