

# 43. Renin Angiotensin

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## Renin Angiotensin Aldosterone System (RAAS) Profile of Administration of 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 the aorta and kidney. It is a well-established fact that chlorogenic acid and anthocyanin found in purple sweet potato generates bioactive compound with antihypertensive and anti-ACE activities in RAA system. The present study sought to investigate anti-ACE activities in RAA system 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 also chlorogenic acid (CA) of 700 mg/kg bw for 4 weeks. Activity of renin, ACE, and Ang II concentration were assessed. Inhibiting activity of renin, ACE, and decreasing Ang II concentration after treatment was observed in the DOCA-salt hypertensive rats compared to normotensive group rats; ( $P < 0.05$ ). This is the first report that demonstrates the lowering of blood pressure and anti-ACE in RAA-system 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 (Fig. 1) 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 antioxidants 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 systole blood pressure (SBP), and activity renin, ACE, and Ang II concentration we used DOCA-salt-induced hypertension Wistar rat (*Rattus norvegicus*) strains as an animal model. The current study demonstrated a decrease in SBP, inhibition of activity renin and ACE also reduction of Ang II concentration after a single oral administration of purple sweet potato ethanol extract in DOCA-salt-induced hypertension rats.

## Materials and methods

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 received, by oral administration, using a canula, 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.

27 Preparation of purple sweet potato ethanol extract (EP)

Purple sweet potatoes were sorted and weighed

27 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 Whatmann paper@40 until getting the filtrate for evaporating and their residue extracting again until 4 times, which was used for the further experiments.

### Analysis of ACE, renin, and angiotensin II of blood serum

Analysis of ACE, renin, and angiotensin II was performed using ELISA kits. The standard, blanks and samples were put into a well of 50  $\mu$ L (except well blank), homogenized, incubated for 30 min at 37°C and washed with 200  $\mu$ L wash buffer added at each well (repeated 5 times, until all liquid was lost). As much as 50  $\mu$ L HRP-Conjugate at every well was then added (except well blank) and followed by homogenization. The plate was closed tightly and incubated for 30 minutes at 37°C. Washing was done with 200  $\mu$ L wash buffer at each well (repeated 5 times, until all liquid was lost). Each well was then added with 50  $\mu$ L chromogen solution A and 50  $\mu$ L chromogen solution B. The plates were tightly closed and incubated for 10 min at 37°C, during which incubation is avoided from light. Furthermore, 50  $\mu$ L stop solution was added at each well (when 4 well containing highest standard concentrations have changed color to blue, if the color change was not uniform, homogenization with shaker was conducted). Performance measurement of absorbance at  $\lambda$  450 nm in the range of time 15 minutes quickly was done using Elisa reader (modification Campbell *et al.*, 1999 and Santiago *et al.*, 2010).

### Analysis of iNOS expression

Cardiac tissue were stored in PFA (paraformaldehyde) 10% and then sectioned and attached on object glass (slides) before de-paraffinized and re-hydrated. Slides later was washed by PBS (Phosphate Buffer Saline) solution then added using Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) and incubated for 20 minutes before washed by addition of PBS. Blocking was performed using addition of BSA (Bovine Serum Albumin 5% (w/v)) in PBS solution and incubated for 30 minutes at room temperature. Slides was washed using PBS solution and then added by iNOS antibody as primary antibody. Slides later incubated with addition of secondary antibody-biotin labelled and then added using SA-HRP (Strep-Avidin Horse Radish Peroxidase) and DAB (diamino benzedine) solution respectively. Counterstaining was performed by addition of Mayer Hematoxylin before being closed by cover glass. Slides was observed under the microscope and later was analyzed by Immunoratio software.

### Statistical Analysis

The results of activity of Renin, ACE and Ang II concentration measurements were expressed as means  $\pm$  standard deviation (SD). Differences between trial groups were statistically analyzed using analysis of variance (ANOVA), followed by the posthoc Tukey test



for determining significant differences at  $p < 0.05$ .

## Result and Discussion

### Renin Angiotensin Aldosterone System (RAAS) Profile

The research was done to determine the effect of purple sweet potatoes and chlorogenic acid to decrease of blood pressure as ACE inhibitor function. To determine whether the renin angiotensin aldosterone (RAAS) system still had an effect on blood pressure regulation for DOCA-salt induced hypertension model, ACE and renin activity were tested and angiotensin II (Ang-II) concentrations against normotensive (NTN), hypertensive of rats and hypertensive (HTN) which were given ethanol extract of purple sweet potato (EP) and chlorogenic acid (CA).

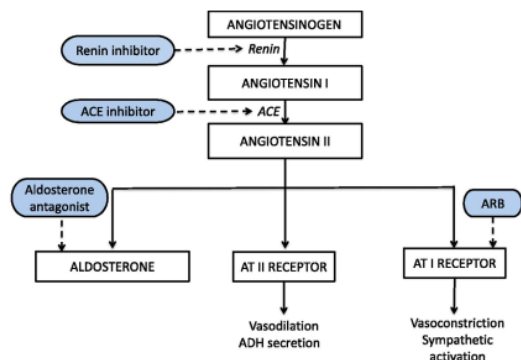


Figure 1. RAAS (Renin Angiotensin Aldosterone System) (Atlas, 2007)

The results of the study (Table 1) showed that high renin activity in the treatment of hypertension ( $554.8 \pm 7.11$  U/L) and slightly decreased after treatment using purple sweet potato extract with 400 mg/kg ( $312.2 \pm 5.94$  U/L) and chlorogenic acid with 700 mg/kg ( $314.0 \pm 6.61$  U/L). Similarly, ACE activity was higher in the hypertensive rats group ( $19.37 \pm 0.90$  U/L) when compared with normal control rats ( $15.03 \pm 0.74$  U/L) and hypertensive rats treated with ethanol extract of purple sweet potatoes (EP) and chlorogenic acid (CA). For concentration of Ang II that resulted of ACE activity which also increasing in hypertension rats ( $913.77 \pm 13.77$  pg/ml) and decreasing after treatment of EP ( $805.29 \pm 11.75$  pg/mL) and CA ( $794.82 \pm 18.01$  pg/mL).

Table 1. Effect of ethanol extract of purple sweet potato (EP) and chlorogenic acid(CA) on ACE activity, Renin and Ang-II

Treatments	Renin(U/L)	ACE (U/L)	Ang II (pg/mL)
K-(Normotensive-NTN)	$303.6 \pm 5.54^a$	$15.03 \pm 0.74^a$	$788.94 \pm 9.52^a$
K+(Hypertensive-HTN)	$554.8 \pm 7.11^c$	$19.37 \pm 0.90^c$	$913.77 \pm 13.77^c$
P1 (HTN +EP 200)	$404.8 \pm 8.40^b$	$18.24 \pm 0.35^b$	$851.88 \pm 7.12^b$
P2 (HTN +EP 400)	$312.2 \pm 5.94^a$	$15.95 \pm 0.40^a$	$805.29 \pm 11.75^a$
P3 (HTN +CA 700)	$314.0 \pm 6.61^a$	$15.89 \pm 0.20^a$	$794.82 \pm 18.01^a$

<sup>a</sup>Values not sharing a common superscript differ significantly at  $P < 0.05$

In hypertensive conditions, there was an increase in activity of Renin, ACE, and levels of Ang II were quite high and decreased after being given ethanol extract of purple sweet potato (EP). The results of this study explain that renin angiotensin system still plays role in blood pressure regulation of rat animal of DOCA-salt

model with purple sweet potato ethanol extract (EP) and chlorogenic acid (CA) containing bioactive compound with ACE-inhibitor activity high enough to inhibit the exchange of Ang I to Ang II. ACE-inhibitor which is a potent vasoconstrictor, so that the blood pressure is not get elevated and beside that, production of ROS also become declined. Because, apart as a potent vasoconstrictor, angiotensin II also contributes to stimulate the production of superoxide in large quantities. The decline in production of free radicals causes reduction level of oxidative stress, thus the endogenous antioxidants could provide therapeutic effect to the body (delMar Contreras *et al.*, 2009).

Table 2 showed that ethanol extract of purple sweet potato (EP) therapy significantly decreased iNOS expression ( $p < 0.05$ ) in rat's cardiac tissue. ACE-inhibitor from ethanol extract of purple sweet potato EP would inhibit activity of ACE enzyme, resulting no conversion from angiotensin I to angiotensin II, which causes decreasing in production of ROS due to inactivated NOX. In positive hypertension group, high production of ROS as result from the activation of NOX will activate NF- $\kappa$ B to encoding iNOS gene (Hong *et al.*, 2000). The increasing expression of iNOS will lead to excess formation of NO (Nitrogen Oxide) and later could bind with superoxide radicals ( $O_2^-$ ) to form peroxynitrite radicals ( $ONOO^-$ ) which is a very reactive cytotoxic substances. The increasing expression of iNOS also produces superoxide radicals ( $O_2^-$ ) which can transform to hydrogen peroxide ( $H_2O_2$ ) spontaneously or by catalytic reaction with superoxide dismutase (SOD). It has been known that the presence of peroxynitrite radicals and hydrogen peroxide has implication to tissue damage and organ dysfunction, including the heart (Sun *et al.*, 2009). According to the previous research of Sun *et al.* (2005), although it was known that the inhibition of iNOS did not have a directly effect on lowering blood pressure and left ventricular hypertrophy, however, the inhibition and lowered expression of iNOS was able to give therapeutic effect on left ventricular contractile tissue caused by decreased level of oxidative stress, as well known that angiotensin II are able to increase the iNOS expression and oxidative stress which causes reducing performance and function of the heart.

## Conclusion

Assessment of the antihypertension properties of purple sweet potato ethanol extract-derived chlorogenic acid in the present study showed a significant effect in inhibiting activity of renin, ACE, Ang II concentration and attenuating abdominal aorta damages in a DOCA-salt hypertensive rat model. These findings suggest that purple sweet potato ethanol extract-derived chlorogenic acid might be considered as a potential antihypertension 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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