Hibiscus sabdariffa Extract Alleviates Insulin Resistance and Blood Pressure in Insulin Resistance Rats Induced by a High Fructose Diet

สารสกัดจากกระเจี๊ยบแดงลดภาวะดื้ออินซูลินและความดันเลือด ในหนูแรทที่มีภาวะดื้ออินซูลินเนื่องจากได้รับอาหารที่มีน้ำตาลฟรุกโทสสูง

Sarawoot Bunbupha (สราวุธ บรรบุผา)* Dr.Poungrat Pakdeechote (ดร.พวงรัตน์ ภักดีโชติ)**
Dr.Upa Kukongviriyapan (ดร.ยุพา คู่คงวิริยพันธุ์)*** Dr.Patchareewan Pannangpetch
(ดร.พัชรีวัลย์ ปั้นเหน่งเพ็ชร)**** Dr.Parichat Prachaney (ดร.ปาริฉัตร ประจะเนย์)*****
Thewarid Berkban (เทวฤทธิ์ เบิกบาน)****** Dr.Arunporn Itharat (ดร.อรุณพร อิฐรัตน์)*******

ABSTRACT

This study aimed to investigate the effect of *Hibiscus sabdariffa* (HS) extract on a insulin-resistant state and blood pressure (BP) in rats with insulin resistance (IR) induced by a high fructose diet for 14 weeks. Fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) were measured. IR rats were either fed with HS extract (500 mg/kg/day) or gallic acid (20 mg/kg/day) while control rats received distilled water (DW) for 4 weeks. BP, FBG, OGTT and vascular functions were tested. Both HS extract and gallic acid improved IR, reduced BP and vascular functions (p<0.05) in IR rats. Conclusions, HS extract alleviated IR, BP and improved vascular functions in IR rats, the mechanism of which may involve its antioxidant effects.

บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดกระเจี๊ยบแดงต่อภาวะดื้ออินซูลินและความดันเลือดในหนูแรทที่มีภาวะดื้ออินซูลินที่ถูกชักนำด้วยอาหารที่มีน้ำตาลฟรุกโทสสูงเป็นเวลา 14 สัปดาห์ โดยทำการวัด Fasting blood glucose (FBG) และ oral glucose tolerance test (OGTT) เพื่อทดสอบภาวะดื้อต่ออินซูลิน ทำการป้อนสารสกัดกระเจี๊ยบ ขนาด 500 มก./กก., gallic acid 20 มก./กก. โดยกลุ่มควบคุมถูกป้อนน้ำเป็นเวลา 4 สัปดาห์ แล้ววัดความดันเลือด, FBG, OGTT และทดสอบการทำงานของหลอดเลือด ผลการทดลองพบว่าการให้สารสกัดกระเจี๊ยบหรือ gallic acid ลดภาวะดื้ออินซูลิน ลดความดันเลือด และปรับการทำงานหลอดเลือดในหนูที่มีภาวะดื้ออินซูลิน (p<0.05) สรุปได้ว่าการให้สารสกัดจากกระเจี๊ยบแดงในหนูแรทที่มีภาวะดื้ออินซูลินสามารถลดภาวะดื้ออินซูลิน ลดความดันเลือดและปรับการทำงานของหลอดเลือดได้ โดยกลไกที่เกี่ยวข้องอาจเนื่องจากฤทธิ์ต้านออกซิเดชันของสารสกัดกระเจี๊ยบแดง

Key Words: Insulin resistance, Hypertension, *Hibiscus sabdariffa*
คำสำคัญ: ภาวะดื้ออินซูลิน ภาวะความดันเลือดสูง กระเจี๊ยบแดง

* *Student, Master of Science Program in Medical Physiology, Faculty of Medicine, Khon Kaen University, Thailand*
** *Assist. Prof. Department of Physiology, Faculty of Medicine, Khon Kaen University, Thailand*
*** *Assoc. Prof. Department of Physiology, Faculty of Medicine, Khon Kaen University, Thailand*
**** *Assoc. Prof. Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Thailand*
***** *Assist. Prof. Department of Anatomy, Faculty of Medicine, Khon Kaen University, Thailand*
****** *Student, Master of Science in Anatomy, Faculty of Medicine, Khon Kaen University, Thailand*
******* *Assoc. Prof. Applied Thai Traditional Medicine Center, Thammasart University (Rangsit Campus)*

Introduction

Insulin resistance (IR) is the condition in which normal amounts of insulin are inadequate to produce a normal response from fat, muscle and liver cells (Bloomgarden, 2007). An insulin-resistant state is normally found in diabetes and obesity, that enormously contributed to hypertension (Reaven, 1995). There are several studies showing that insulin resistance plays an important role in pathogenesis of hypertension. For example, insulin increased sympathetic activity in skeletal muscle of patients with hypertension (Lembo et al., 1992). Potenza and coworkers (2009) suggested that insulin resistance and hyperinsulinemia induced endothelial dysfunction and developed to hypertension (Potenza et al., 2009).

Hibiscus sabdariffa (HS), commonly known in English as roselle or red sorrel, has been generally used as soft drinks and medicinal herbs. Recent studies also been demonstrated its beneficial effect as the antihypertensive**,** antioxidant, anti-obesity and hypocholesterolemic activities (Ajay et al., 2007; Hirunpanich et al., 2006)**.** In addition, Yosaph and coworkers (2009) found that the water extract of *H. sabdariffa* reduced blood glucose and serum insulin level in high fructose-high fat diet induced diabetic rats (Yosaph et al., 2009). However, little information is known the therapeutic effects of HS extract on blood pressure and vascular responsiveness in hypertensive rats with insulin resistance induced by a high fructose diet (HFD). Therefore, the present study is aimed to examine the effect of HS extract on insulin resistant status, blood pressure and vascular functions in hypertensive rats with insulin resistance-induced by a HFD.

Materials and methods

Plant extract

The HS extract was prepared using water extract and supplied by Assoc. Prof. Arunporn Itharat, Applied Thai Traditional Medicine Centre, Thammasart University, Prathumthani. Quantitative determination of active compounds of HS extract by HPLC found that it composed of quercetin 0.50 mg/g, gallic acid 0.15 mg/g and cyanidin-3-glucoside 2.74 mg/g (Chaovanalikit et al., 2004).

Animals and experimental protocol

Male Sprague-Dawley rats (120-140 g) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were maintained in an air-conditioned room (25±1° C) with a 12 h dark-light cycle at Northeast Laboratory Animal Center**.** All procedures are complied with the standards for the care and use of experimental animals and approved by Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (AEKKU 41/2551)**.**

After one week of acclimatization, the animals were randomly divided into 4 groups (n = 10/group). Group 1, the normal control group, received normal diet (C.P. Mice feed 082) and distilled water (DW) throughout an experimental period, whereas in other groups (Group 2-4), rats were fed with a HFD for 14 weeks to induce insulin-resistant condition and DW was available *ad libitum*. The HFD composed of 60% fructose, 20% casein, 10% palm oil and 10% other supplemented minerals (Guo et al., 2007; Reeves et al., 1993). After 14 weeks of HFD treatment, normal rats were orally administered with DW and IR rats, group 2, 3 and 4, were orally given DW, HS extract (500 mg/kg/day), gallic acid (20

mg/kg/day) respectively for 4 weeks**.** Gallic acid is a type of phenolic compounds, derived from natural products and also found in HS extract. It is well know as a powerful antioxidant agent (Kim, 2007). Rats received gallic acid was a positive control group. The choice of concentration of HS extract used in this study, 500 mg/kg was followed by previous study (Onyenekwe et al., 1999).

Indirect measurement of blood pressure

Animals were determined systolic blood pressure (SP) using a tail-cuff plethysmography (IITC model 179 blood pressure analyzer) once a week to assess blood pressure changes during 4 weeks of treatments.

Fasting blood glucose and oral glucose tolerance test assessments

The FBG was investigated at the end of 14 weeks of HFD or normal diet feeding and measured again at the end of further 4 weeks of treatments. In procedure, rats were fasted overnight (8-10 hr) and blood samples were taken from lateral tail vein to examine the FBG using a glucometer (ACCU-CHEK Advantage, USA). Then, rats were orally administered with glucose at dose of 2 g/kg in order to determine glucose tolerance. In this experiment, the blood glucose concentrations at before glucose loading (0 min) and at 30 and 120 min after glucose administration were investigated. Thereafter, area under the curve (AUC) of OGTT was calculated according to the formula of Tai's model (Tai, 1994).

Hemodynamic and vascular reactivity assessments

At the end of experiment, the animals were anesthetized by peritoneal injection of pentobarbital-sodium (60 mg/kg) and placed on heating pad. Subsequently, a tracheotomy was made to assist respiration. The femoral artery was identified, cleaned of connective tissue and cannulated with a polyethylene tube. SP, diastolic blood pressure (DP), mean arterial blood pressure (MAP) and heart rate (HR) were continuously monitored by a way of a pressure transducer and recorded using the Acknowledge data acquisition with analysis software (Biopac System Inc., California, USA.).

Femoral vein was also cannulated with a polyethylene tube. To test the endothelial and smooth muscle cell functions, a vascular responsiveness was carried out by intravenous infusion of acetylcholine (ACh), an endothelium dependent vasodilator, (3, 10, 30 nmol/kg), sodium nitroprusside (SNP), an endothelium independent vasodilator, (1, 3, 10 nmol/kg) and phenylephrine (Phe), an α_1 adrenoceptor agonist, (0.01, 0.03, 0.1 μmol/kg).

Statistical analysis

Results were expressed as mean ± S.E.M. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests. A *p*-value of less than 0.05 was considered a statistical significance.

Results

Effect of HS extract and gallic acid on insulin resistant status

Rats' body weight did not differ among all groups. Feeding of HFD for 14 weeks significantly increased FBG levels (IR rats; 110.3 ± 1.9 mg/dl, control rats; 80.8 ± 1.4 mg/dl) and AUC of OGTT (IR rats; 16,265 ± 368.9 mg/dl/120 minutes, control rats; 13,260 ± 522.5 mg/dl/120 minutes) ($p<0.05$) comparing to those of rats fed with a normal diet. Increasing FBG levels in IR rats was attenuated by HS

extract (500 mg/kg/day) or gallic acid (20 mg/kg/day). Similar results were demonstrated with AUC of OGTT when there were a significant improvement of insulin-resistant state in IR rats received either HS extract or gallic acid (Figure 1).

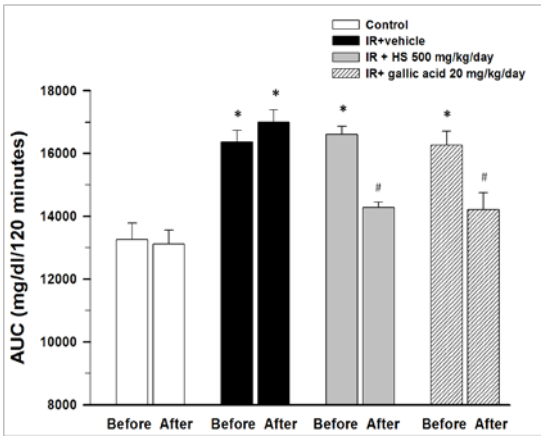

Figure 1 Area under the curve (AUC) of oral glucose tolerance tests before and after treatment in control rats, IR rats, IR rats received HS extract (500 mg/kg/day) and IR rats received gallic acid (20 mg/kg/day) * $p < 0.05$ vs. control, # $p < 0.05$ vs. before HS extract or gallic acid treatment, (n = 6/group).

Effect of HS extract and gallic acid on blood pressure (indirect measurement)

Indirect blood pressure showed a significant increase in SP (144.46 ± 3.8 mmHg vs. 118.78 ± 2.1 mmHg) (p<0.001) after 14 weeks of HFD feeding when comparing to those of normal control rats. However, treatments of HS extract 500 mg/kg/day for 4 weeks decreased SP (130.50 ± 1.2 mmHg vs. 152.54 ± 3.2 mmHg) in IR rats when compared to those of IR rats without treatment. In addition, a reduction of SP (123.30 ± 1.8 mmHg) in IR rats treated with gallic acid for 4 weeks was also observed (Figure 2).

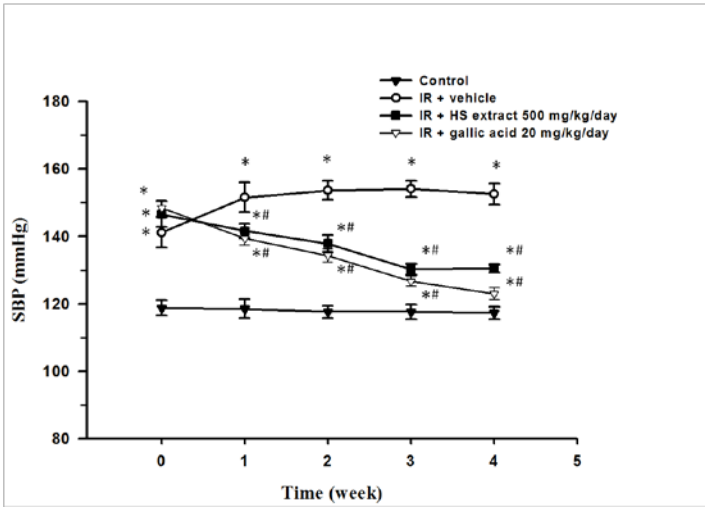

Figure 2 Effects of HS extract (500 mg/kg/day) and gallic acid (20 mg/kg/day) on SP (indirect measurement) in control group, IR group, IR received HS extract group and IR received gallic acid group. * $p < 0.05$ vs. control, # $p < 0.05$ vs. IR + vehicle, (n = 8-10/group).

Effects of HS extract and gallic acid on SP, DP, MAP and HR

Similar BP results were found with direct method that there were significant increases in SP, DP and MAP (p<0.05) in rats fed with HFD. The change of HR did not observed in all group of rats. Interestingly, a concomitants administration of HS extract for 4 weeks significantly reduced SP, DP and MAP in IR rats. Moreover, daily treatment with gallic acid (20 mg/kg/day) for 4 weeks also significantly decreased SP, DP and MAP (p<0.05) when compared to those of IR rats. Neither HS extract nor gallic acid had effect on HR (Table 1).

Table 1 Effects of HS extract and gallic acid on SP, DP, MAP and HR in all experimental groups.

Parameters	Normal control	IR+ vehicle	IR+ HS extract 500 mg/kg/day	IR+ gallic acid 20 mg/kg/day
SP (mmHg)	121.85 ± 3.6	157.23 ± 2.2*	135.44 ± 2.4$^{*\#}$	126.72 ± 1.8$^{\#\dagger}$
DP (mmHg)	79.16 ± 2.8	104.41 ± 2.0*	85.56 ± 1.4$^{\#}$	81.25 ± 1.7$^{\#}$
MAP (mmHg)	93.39 ± 3.0	122.02 ± 1.9*	102.19 ± 1.6$^{*\#}$	96.41 ± 1.4$^{\#}$
HR (beats/min)	347.06 ± 7.2	371.88 ± 7.0	356.76 ± 8.5	365.6 ± 13.6

* $p < 0.001$ vs normal control, # $p < 0.05$ vs IR + vehicle, † $p < 0.05$ vs IR + HS extract 500 mg/kg/day, n = 7-9/group

Effect of HS extract and gallic acid on a vascular responsiveness

Vasodilation responses to ACh (3, 10, 30 nmol/kg) was significantly impaired in IR rats received DW when compared to that of normal control rats (30.80 ± 1.1 % vs. 36.22 ± 1.2 %), (43.06 ± 1.5 % vs. 47.99 ± 1.2 %) and (49.21 ± 0.5 % vs. 57.08 ± 1.2 %) dose respectively. Treatments of either HS extract (500 mg/kg/day) or gallic acid (20 mg/kg/day) for 4 weeks in IR rats markedly restored the vasodilation response to ACh at 30 nmol/kg (p<0.05). However, there was no difference in vascular responses to Phe and SNP in all groups of rats (Figure 3).

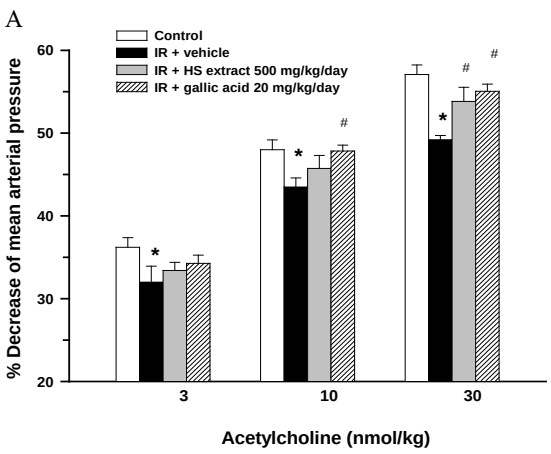

Figure 3 Effect of HS extract (500 mg/kg/day) and gallic acid (20 mg/kg/day) on vascular responsiveness in insulin-resistant rats. Acetylcholine (A), Sodium nitroprosside (B), and Phenylephrine (C). *p < 0.05 vs. control, #p < 0.05 vs. IR + vehicle. (n = 8-10/group)

Discussion

This present findings showed that feeding of HFD in rats for 14 weeks led to an insulin-resistant condition and hypertension. Since there was an increase in FBG levels, an impairment of OGTT in rats received HFD. Increased blood pressure and a blunted vascular response to ACh were found in this animal model. Administration of either HS extract or gallic acid evidently alleviated insulin resistant status, decreased blood pressure which was associated with an improvement of vascular endothelial functions.

HFD feeding caused an increase in FBG levels and impaired OGTT, which indirectly indicated insulin-resistant status in rats. Induction of insulin resistance using a HFD has been reported that intake of a HFD led to increased FBG, impaired of OGTT and developed insulin resistance in rats (Bocarsly et al., 2010; Suwannaphet et al., 2010). The mechanisms of HFD induced insulin resistance may involve the reduction of the insulin-stimulated IRS-1 as well as PI3-kinase activity in liver and muscle cells (Bezerra et al., 2000). In addition, a rise in blood pressure has been found in HFD model (Karthik et al., 2011). This observation was consistent with our results that HFD induced hypertension.

We have known that blood pressure is determined by cardiac output and total peripheral resistance. A rise in blood pressure of this animal model has been reached mild hypertension. A blunted vascular response to ACh was also observed in IR rats while vascular response to SNP and Phe did not alter in this animal model. These results could imply that there was only an impairment of vascular endothelial function in IR rats. These endothelial vascular dysfunctions cause an imbalance of vascular tone and an increase in total peripheral resistance, while the alterations of HR in IR rats did not showed. The mechanism of insulin resistance related to hypertension is controversy. One possible mechanism is that insulin resistance induced endothelial dysfunction by decreasing eNOS expression and nitric oxide (NO) production (Potenza et al., 2009). Furthermore, insulin-resistant state and diabetes are associated to reduced endothelium-dependent relaxation and linked to cardiovascular events (Villalba et al., 2009).

This result showed that HS extract treatment lowered FBG and improved an OGTT in HFD feeding rats. Similarly, the blood glucose level in alloxan-induced diabetic rats was decreased significantly after 4 weeks of HS extract treatment that was associated with its antioxidant capacity (Farombi and Ige, 2007). In addition, Yosaph and colleagues (2009) also found the improvement of OGTT and insulin resistance in high fat and fructose diet feeding rats (Yosaph et al., 2009). The HS extract recovered blood glucose regulation in this study accompanying with a reduction of blood pressure in HFD feeding rats. Indicating, antihypertensive effects of HS extract in a HFD induced insulin resistance rats. Several lines of evidence supported that *H. sabdariffa* could act as antihypertensive agent. In rat 2-kidney, 1-clip renovascular hypertension model, chronic administration of *H. sabdariffa* was able to decrease systolic blood pressure which was associated with a reduction of heart weight and heart rate (Odigie et al., 2003). However, the antihypertensive effect of HS extract in the present study involved the improvement of vascular function since the decreased vascular response to ACh was recovered in IR rats after treatment with HS extract. This result supported by

Ajey and co-workers (2007). They found that anti-hypertensive effect of the calyces of *H. sabdariffa* related to endothelium-dependent vasorelaxation in isolated aortas from spontaneously hypertensive rats (Ajay et al., 2007).

Under insulin-resistant conditions found an increase in oxidative stress which lead to endothelial dysfunction associated with lack of NO bioavailability (Schulz et al., 2008). HS extract has been reported to have an antioxidant activity as it contains high riches of anthocyanins and gallic acid (Liu et al., 2002). This study we use gallic acid as positive control and the result showed that gallic acid had the beneficial effects including, reduced FBG levels, AUC of OGTT, blood pressure and improved vascular responsiveness in hypertensive rats with insulin resistance. Gallic acid is a phenolic compound, it is a major component in *Hisbicus Sadarrifa* and reported to have potential antioxidant effect such as ability to scavenge free radicals and increased nitric oxide availability (Punithavathi et al., 2011).

Conclusions

Our study demonstrated that HS extract reduced FBG levels, AUC of OGTT, blood pressure and improved vascular responsiveness in hypertensive rats with insulin resistance. The underlying mechanisms are likely to be related to its antioxidant capacity. This finding supports the beneficial effect on the cardiovascular system related to insulin resistance of a *Hisbicu sabdariffa*, which is widely consumed in several countries.

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