Hibiscus sabdariffa Extract Ameliorates Insulin Resistance and Increases Aortic Compliance in High-Fructose-Fed Rat Model

สารสกัดกระเจี๊ยบแดงลดภาวะดื้ออินซูลินและเพิ่มความยืดหยุ่นของหลอดเลือดแดงใหญ่เออร์ตา ในหนูแรทที่ได้รับอาหารที่มีน้ำตาลฟรุกโทสสูง

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ABSTRACT

We determined the effect of *Hibiscus sabdariffa* extract (HSE) on insulin resistance, hypertension, and elastic property of the thoracic aorta in rats induced insulin resistance (IR) by high-fructose diet (HFD) for 14 wks. Fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) were measured to assess the IR conditions. The IR rats were divided into two groups, IR+vehicle and IR+HSE (500 mg/kg/day). Blood pressure (BP), heart rate (HR) and thoracic elasticity were investigated. The increase in FBG, BP, and HR, in IR rats were significantly reduced by HSE (p<0.05). The IR+HSE showed less functional elastic modulus than those of control and IR rats at the pressure higher than 80 mmHg (p<0.05), suggesting an increase in the aortic compliance effect of HSE. Conclusion, the HSE improved IR, reduced BP and increased elasticity of conduit artery in IR rats induced by HFD.

บทคัดย่อ

เราศึกษาผลของสารสกัดกระเจี๊ยบแดงต่อภาวะดื้ออินซูลิน ความดันเลือดสูง และการเปลี่ยนแปลงคุณสมบัติความยืดหยุ่นของหลอดเลือดแดงใหญ่ในหนูแรทที่มีภาวะดื้ออินซูลินเนื่องจากได้รับอาหารที่มีน้ำตาลฟรุกโทสสูงเป็นเวลา 14 สัปดาห์ ประเมินภาวะดื้ออินซูลินโดยวัดระดับ fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) แบ่งหนูทดลองที่มีภาวะดื้ออินซูลินออกเป็นสองกลุ่ม คือ กลุ่มภาวะดื้ออินซูลิน และกลุ่มภาวะดื้ออินซูลินที่ได้รับสารสกัดกระเจี๊ยบขนาด 500 มล./กก. พบว่าสารสกัดกระเจี๊ยบ ลดการเพิ่มขึ้นของ FBG ความดันเลือดและอัตราการเต้นของหัวใจในหนูกลุ่มที่มีภาวะดื้ออินซูลินอย่างมีนัยสำคัญ (p<0.05) และหนูกลุ่มนี้ยังมีค่ามอดูลัสของสภาพความยืดหยุ่นน้อยกว่ากลุ่มอื่นอย่างมีนัยสำคัญ (p<0.05) ในระดับความดันที่มากกว่า 80 มิลลิเมตรปรอท แสดงให้เห็นว่าสารสกัดกระเจี๊ยบแดงเพิ่มความยืดหยุ่นของหลอดเลือด โดยสรุปสารสกัดกระเจี๊ยบแดงสามารถลดภาวะดื้ออินซูลิน ลดความดันเลือด และ เพิ่มยืดหยุ่นของหลอดเลือดแดงใหญ่ ในหนูที่มีภาวะดื้ออินซูลินที่ถูกชักนำด้วยอาหารที่มีน้ำตาลฟรุกโทสสูง

Key Words: Insulin resistance, *Hibiscus sabdariffa* L., Arterial elasticity

คำสำคัญ: ภาวะดื้ออินซูลิน กระเจี๊ยบแดง ความยืดหยุ่นของหลอดเลือดแดง

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Introduction

Insulin resistance (IR) is a condition in which the cells of the body, particularly, fat cells, muscle cells and liver cells do not appropriately respond to insulin. Several studies suggested that the insulin resistance plays an important role to vascular disease such as artherosclerosis (Mule et al., 2006; Noma et al., 1999; Scuteri et al., 2004) and arterial stiffness (Cameron and Cruickshank, 2007; van Popele et al., 2000; Yki-Jarvinen and Westerbacka, 2007). Collagen and elastin are the main component in the large artery. Changing in these components properties as a result of increasing in advanced glycation end-products (AGE) has been reported (Verzijl et al., 2000; Winlove et al., 1996). In addition, AGE is also known to enhance the generation of reactive oxygen species (ROS) (Rojas et al., 2000). It has been reported that accumulation of AGE is found in various tissue of rats with high fructose intake including aorta. Recently, our group reported that antioxidant compound improves arterial elasticity in hypertensive rats with reduction of oxidative stress (Nakmareong et al., 2011).

Hibiscus sabdariffa Linn. (*H. sabdariffa*), (in Thai; Krajiab Dang) has many biological activities such as antihypertension (Mozaffari-Khosravi et al., 2009), antihyperlipidemia (Gurrola-Diaz et al., 2010), antiobesity (Yosaph et al., 2009), antiatherosclerosis (Chen et al., 2003) and antioxidant (Hirunpanich et al., 2005). However, less is known about therapeutic effect of *Hibiscus sabdariffa* extract (HSE) on aortic elasticity in high fructose diet induced insulin resistance rats. Therefore, the present study is aimed to investigate the effect of HSE on insulin resistance, blood pressure and elastic property of the thoracic aorta in high fructose diet-induced insulin-resistant rats.

Materials and methods

Preparation of HSE

Fresh calyces of *H. sabdariffa* were dried at 50°C for 24 hours. The dried calyxes of *H. sabdariffa* (2 kg) were blended and boiled with water (1:20) at 60°C for 60 minutes, then the water extract was filtered and dried using spray dry machine. By this procedure the yield is 37.4%. Dried *H. sabdariffa* extract was then packed in tight containers and kept at 4-6°C.

Animals

Male Sprague-Dawley rats weighing 120-140 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were housed in stainless steel cages and maintained in controlled conditions (temperature: 25.1±1˚C and a 12 h dark-light cycle). The procedures were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 43/2551).

Experimental design and insulin resistant induction

After seven days of acclimatization, the animals were randomly divided into 3 groups with 10 rats per experimental group. Group 1,the normal group was fed with a standard chow diet (Chareon Pokapan Co., Thailand) and distilled water throughout an experimental period, whereas in other the two groups (Group 2 and 3), rats were fed with a high fructose diet (HFD) for 14 weeks to induce insulin-resistant condition and water was available *ad libitum*. After 14 weeks of IR induction, normal control rats were orally administered with distilled water which IR rats, group

2 and 3 were orally given distilled water or HS extract (500 mg/kg/day) for further 4 weeks.

Fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) assessments

To assess the development of the insulin resistance, the FBG and OGTT were examined at the end of 14 weeks of high-fructose and normal diet feeding and reexamined at the end of the further 4 weeks of treatments. Rats in each group were randomized into 6 and were fasted overnight (8-10 hr). Then blood samples were taken from tail 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 (OGTT). 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 2006 American Diabetes Association (Association., 2006).

Indirect measurement blood pressure

Indirect blood pressure were measured once a week at the beginning of the 15th weeks until the end of the experiment. Systolic blood pressure (SBP) were measured in conscious rats by using the indirect method of tail-cuff plethysmography (Blood pressure analyzer, model 29; IITC, Woodland Hills, California, USA) to assess the development of the insulin resistance during HSE treatment.

Direct measurement blood pressure

At the end of the experiment, rats were anaesthetized with an intraperitoneal injection of Pentobarbital (60 mg/kg). A tracheotomy were performed for spontaneous of breathing, and a polyethylene catheter were inserted into the lower abdominal aorta via the left femoral artery to connect with a pressure transducer for continuous monitoring of blood pressure using the Acqknowledge data acquisition and analysis software (BIOPAC Systems Inc., California, USA). Baseline blood pressure values and heart rate were monitored for 10 minute of each animal.

Aortic elasticity measurement

The elastic properties of the thoracic aorta were measured in situ by diameter changed of vessel, while pressurizing to the vessel. To determined vascular elasticity, the rats were open the thoracic wall to expose the thoracic aorta. The polyethylene catheters were inserted into the descending thoracic aorta at distal to arch of aorta and another catheter were inserted into the descending thoracic aorta below the diaphragm. The normal salines were flush at lower catheter of the thoracic aorta to remove any thrombus, and then the barium sulphate apparatus were attached at upper catheter of the thoracic aorta. The animals and the instrument were placed under a camera with a macro lens. Initially, the vessel was pressurized with 200 mmHg for 30 seconds in order to approach a preconditioning condition. After deflation the aorta will be extend to pressure of 0, 10, 20, 30, 50, 70, 90, 110, 120, 140, 160, 180, 200, 220 and 240 mmHg for 2 rounds. Each pressure was maintained for 30 seconds to ensure equilibration of pressure between the perfusion instruments. The vascular images of each pressure were captured and processed with integrated image analysis software (Image-Pro Plus, Maryland, USA) for calculate the external diameter of the vessel (Nakmareong et al., 2011).

Table 1 Effect of HSE on fasting blood glucose (n=6)

	Normal control Group	IR+Vehicle group	IR+HSE 500 mg/kg/day group
FBG (mg/dL)			
Pre-treat	89.67±2.30	100.83±2.39*	97.83±1.08*
Post-treat	80.00±1.46	111.83±2.63*	95.83±2.40*#

IR, Insulin resistance; HSE, *Hibiscus sabdariffa* extract; FBG, Fasting blood glucose

*P<0.05 compared with normal control group #p<0.05 compared with IR+Vehicle group

Statistic analysis

Data will be expressed as mean ± S.E.M. The significance of differences between means was analyzed by one-way analysis of variance (ANOVA) and followed by post-hoc Duncan's multiple range test. Statistical significance will be assigned at a *P* value of less than 0.05.

Results and discussion

Effect of HSE on fasting blood glucose (FBG) and oral glucose tolerance test (OGTT)

FBG and OGTT in the normal control rats, IR rats and IR rats received HSE 500 mg/kg/day of pre-treat and post-treat by distilled water and HSE 500 mg/kg/day respectively were calculated. The values of FBG of pre-treat and post-treat were significantly increased in the IR rats and IR rats received HSE 500 mg/kg/day when compared to the control rats. Moreover, the values of FBG of post-treat were significantly decreased in the IR rats received HSE 500 mg/kg/day when compared to the IR rats, but no significant difference when compared with the control rats (Table 1). The values of area under the curve (AUC) from OGTT of pre-treat and post-treat were significantly higher in the IR and IR rats received HSE 500 mg/kg/day rats when compared to those in the control. In addition, the values of area under the curve (AUC) from OGTT in the IR rats received HSE 500 mg/kg/day were significantly decreased when compared between pre-treat and post-treat in same group with a contrast result was shown in the IR rats. There was no significant difference in AUC from OGTT of pre-treat and post-treat in the control rats (Figure 1).

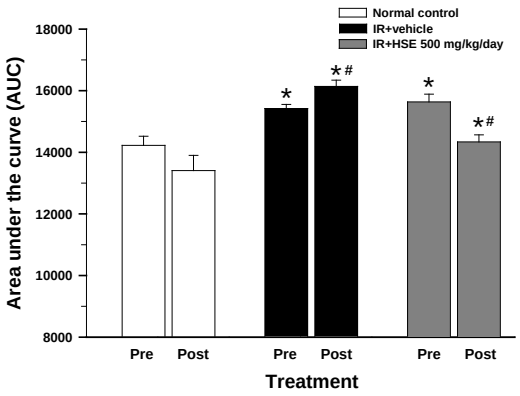

Figure 1 Area under the curve (AUC) from oral glucose tolerance test pre-treat and post-treat in control rats, IR rats and IR rats received HSE 500 mg/kg/day. n=6/group *P<0.05 compared with normal control group #P<0.05 compared with pre-treat in same

Effect of HSE on systolic blood pressure (indirect method)

Systolic blood pressure was calculated at pre-treat, 1, 2, 3 and 4 weeks. Values of SBP significantly increased in the IR rats in pre-treat, 1, 2, 3 and 4 weeks when compared to the control rats. Similar result in the IR rats received HSE 500 mg/kg/day in pre-treat. However, the IR rats received HSE 500 mg/kg/day showed significantly decreased systolic blood pressure at 1, 2, 3 and 4 weeks when compared to IR rats (Figure 2).

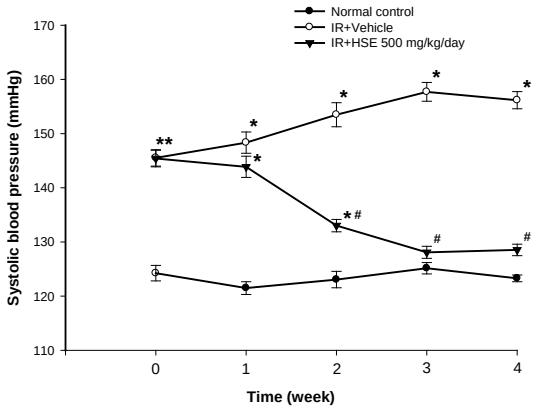

Figure 2 Effect of HSE on systolic blood pressure (n=9-10/group) *P<0.05 compared with normal control group #P<0.05 compared with IR+Vehicle group

Effect of HSE on blood pressure and heart rate (direct method)

Systolic blood pressure (SBP), mean arterial pressure (MAP), diastolic blood pressure (DBP), pulse pressure (PP) and heart rate (HR) of the IR rats significantly increased when compared to those of the control rats. However, the values of SBP, MAP, DBP, and HR in the IR rats received HSE 500 mg/kg/day were significantly decreased when compared to the IR rats (Table 2).

Effect of HSE on Elasticity of the thoracic aorta

The mean relative radius (strain) was plotted against all pressures (stress). At over than 100 mmHg, IR+HSE has higher relative radius compare to those in other groups (Figure 3a) but there is no significant difference between groups. However, the functional elastic modulus (Ep) of IR+HSE is lesser than those in the other two groups (Figure 3b) with a significant difference at pressure over 80 mmHg.

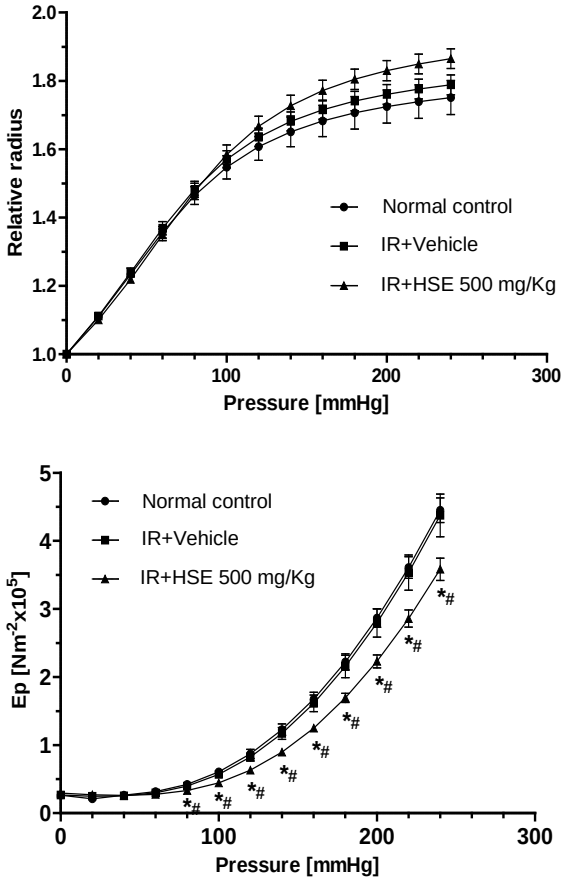

Figure 3 Effect of HSH on relative radius (a), functional elastic modulus (b) (n=5-6/group) *P<0.05 compared with normal control group #P<0.05 compared with IR+Vehicle group.

Conclusions

The main finding of this study is that HSE 500 mg/kg/day has an effectively improve vascular function in insulin resistant rat induced by high fructose intake. This study reveals that high fructose

diet for 14 weeks increased fasting blood glucose, impaired glucose tolerance and increased blood pressure in insulin resistance rats. Treatment of HSE 500 mg/kg/day for 4 consecutive weeks showed much improved in fasting blood glucose and oral glucose tolerance in this model. Similar result was shown in the study of Wisetmuen and coworkers in that. *H sabdariffa* extract at doses of 0.5 and 1.0 g/kg BW/day on produced a significant reduction in the blood glucose of type II diabetic rats (Wisetmuen et al., 2008). Likewise, in high fructose and fat diet feeding-induced type II diabetic rats, the water extract of *H. sabdariffa* at 0.1, 0.5 and 1.0 g/kg BW/day for 4 weeks, significantly decreased the blood glucose (Yosaph et al., 2009).

Table 2 Effect of HSE on Direct blood pressure, pulse pressure and heart rate (n=11)

	Normal control Group	IR+Vehicle group	IR+HSE 500 mg/kg/day group
SBP (mmHg)	124.24±1.79	154.44±1.64*	132.68±1.53*#
MAP (mmHg)	98.95±1.66	124.85±1.47*	105.03±1.31*#
DBP (mmHg)	81.61±1.62	103.79±1.65*	84.10±1.28#
PP (mmHg)	42.26±1.34	50.72±1.73*	48.67±1.21*
HR (bpm)	342.64±11.87	387.71±9.00*	350.49±9.91#

*P<0.05 compared with normal control group #p<0.05 compared with IR+Vehicle group.

In addition, we found that HSE 500 mg/kg/day significantly reduced BP and HR in this animal model. Previous studies have reported that the sour tea producing from *H. sabdariffa* reduced the systolic blood pressure and mean pulse pressure in patients with type II diabetes. (Mozaffari-Khosravi et al., 2009). In addition, Herrera-Arellano and coworkers evaluated the effects of *H. sabdariffa* 9.6 mg anthocyanins content in patients with mild to moderate hypertension for 4 weeks. They found that *H. sabdariffa* decreased the systolic and diastolic blood pressure (Herrera-Arellano et al., 2004). Consistently with Herrera-Arellano finding, the present study demonstrates that HSE 500 mg/kg not only has anti-hypertensive activity but also causes heart-rat-decreasing effect. However, the mechanisms responsible for the blood pressure-lowering affect *H. sabdariffa* have not been fully elucidated. Several studies suggested that anti-hypertensive effect of *H. sabdariffa* was on an improvement of endothelial function (Ajay et al., 2007). This similar result was found in another experiment of our laboratory in IR rats which HSE decreased vascular response to acetylcholine (Ach).

One of the main findings of the present study is that the elastic modulus-stress curve of IR+HSE was significantly shifted rightward compared with that of control and IR+vehicle at the pressure over 80 mmHg. This result indicates a higher elasticity of the thoracic aorta in IR treated with HSE rats than in normal control and IR control rats. Mamadou Sarr and colleagues reported that H. *sabdariffa* induced vasorelaxation in rat thoracic aortic ring (Sarr *et al.*, 2009). Moreover, a major component in *H. sabdariffa* is a phenolic compound which has potential

antioxidant property to serve as a scavenger of several ROS and improve nitric oxide availability (Punithavathi et al. 2011). Surprisingly, there is no significant difference in elastic modulus between control and IR control rats. Although it has been shown the association between IR and arterial stiffness, contrast result was found in this present study. A possibility to explain this controversial finding is that IR induced by high fructose diet for 14 weeks may not sever enough to alter the structure of the large artery. Another possibility is that the thoracic aorta of the IR+vehicle has been compensating since mild hypertension also found in this study. Additionally, it has been reported that in early-stage insulin resistance, nitric oxide and agiotensin II receptor activity reduce arterial stiffness. (Woodman, 2008). Therefore, studying the structural change in large artery, the time induced IR should take into account in this animal model.

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