Hibiscus sabdariffa extract improves insulin resistance and oxidative stress status in insulin-resistant rats induced by a high fructose diet

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Abstract

Rats fed a high fructose diet served as an animal model of insulin resistance which was associated with increase in oxidative stress status. *Hibiscus Sabdariffa* (HS) has been reported to exhibit a potent antioxidant activity. The objective of this study was to investigate the effect of HS extract on an insulin-resistant state and oxidative stress status in rats with insulin resistance (IR) induced by a high fructose diet (HFD) for 14 weeks. The IR rats received HS extract (500 mg/kg), gallic acid (20 mg/kg) and vehicle, while control rats received distilled water (DW) for 4 weeks. Fasting serum insulin, fasting blood glucose (FBG), oral glucose tolerance test (OGTT), and plasma malondialdehyde (MDA) were evaluated. Homeostasis model assessment of insulin (HOMA-IR) was calculated. Results showed that rats received a HFD had high fasting serum insulin, FBG levels, OGTT impairment and a high plasma MDA level (p<0.05). However, HS extract or gallic acid reduced fasting serum insulin, FBG levels, improved OGTT and oxidative stress status (p<0.05) in IR rats. In conclusions, HS extract improved insulin sensitivity related to decrease an oxidative stress marker indicating its antioxidant effects.

Keywords: Insulin resistance, oxidative stress, *Hibiscus sabdariffa*

1. Introduction

Insulin resistance is the condition in which normal amounts of insulin are inadequate to produce a normal response from fat, muscle and liver cells [1]. Oxidative stress is a consequence of the imbalance between reactive oxygen species (ROS) production and antioxidant capacity [2]. There was evidence to show that oxidative stress enhances insulin resistance in several animal models. For example, ROS over-production in the adipose tissues and liver cells in mice lead to the development of obesity and insulin resistance induced by a high-fat diet [3]. In addition, plasma oxidative stress markers were increased in rats with insulin resistance and these can be alleviated by the superoxide dismutase [4].

Hibiscus sabdariffa, commonly known in English as roselle or red sorrel, has been generally used as soft drinks and medicinal herbs. Recent studies also demonstrated its beneficial effects as the antioxidant, antihypertensive, anti-obesity and hypocholesterolemic activities [5, 6]. 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 [7]. Here we have studied the effect of HS extract on insulin sensitivity and oxidative stress status in IR rats induced by a HFD.

2. 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 [8].

Animals and experimental protocols

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 = 8/group). Group 1, the normal control group, received normal diet (C.P. Mice feed 082) and DW throughout an experimental period, whereas in other groups (Group 2, 3 and 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 [9, 10]. 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) and gallic acid (20 mg/kg/day) respectively for 4 weeks.

Fasting blood glucose and oral glucose tolerance test assessments

The FBG was investigated at the end of 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 [11].

Fasting serum insulin assessments and HOMA-IR calculation

The fasting serum insulin was investigated at the end of 4 weeks of treatments with similar protocol as in the OGTT. The concentration of insulin in serum at before (basal secretion) glucose loading was examined using Rat insulin ELISA Kit (Linco, U.S.A.). HOMA-IR score was expressed as an index of insulin resistance [12] and calculated as following [9].

HOMA = (fasting glucose (mmol/l) × fasting insulin (μIU/ml)) / 22.5

3. Assay of plasma malondialdehyde (MDA)

At the end of treatment, rats were anesthetized with peritoneal injection of pentobarbital-sodium (60 mg/kg) and blood samples were collected for plasma MDA measurement. The level of MDA was assayed following a previous described method of Luangaran and coworkers (2007). In brief; 150 μl of plasma was reacted with 10 % TCA, 125 μl of 5 mM EDTA, 125 μL of 8 % SDS and 10 μl of 0.5 μl/ml of BHT. The mixture was left for 10 min, then 0.6 % TBA was added in an equal volume and the mixture was heated for 30 min in a boiling water bath. After cooling to room temperature, the mixture was centrifuged 10,000 g for 5 minutes at 25 °C. The absorbance of the supernatant was measured at

the wavelength of 532 nm by spectrophotometry. A standard curve was generated using appropriate concentrations of standard TEP (0.3-10 μmol/1) [13].

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.

4. Results

Effect of HS extract on insulin resistant status

Rat body weight was similar in all groups. Rats feeding with HFD for 14 weeks significantly increased FBG levels (112.5 ± 1.7 mg/dl) comparing to those of rats fed with a normal diet (82.3 ± 1.3 mg/dl) (p<0.05). Similar results were demonstrated with AUC of OGTT (IR rats; 17,002 ± 396.1 mg/dl/120 minutes, control rats; 13,132 ± 463.2 mg/dl/120 minutes) (Figure 1), and HOMA-IR score (IR rats; 8.76 ± 0.35, control rats; 1.42 ± 0.36) (p<0.05). That indicated insulin-resistant status in rats treated with a HFD (Figure 2). IR rats received HS extract for 4 weeks showed a reduction of FBG (93.9 ± 1.7 mg/dl) and AUC (14,232 ± 134.2 mg/dl/120 minutes). In addition, gallic acid exhibited hypoglycemic effect by significantly decreasing FBG (93.9 ± 1.7 mg/dl) and AUC (14,217 ± 529.1 mg/dl/120 minutes) in IR rats (p<0.05). This was associated with the improvement of insulin sensitivity as expressed by decreasing in HOMA-IR score in rats treated with HS extract or gallic acid (4.91 ± 0.62 and 2.16 ± 0.27 respectively) (p<0.05).

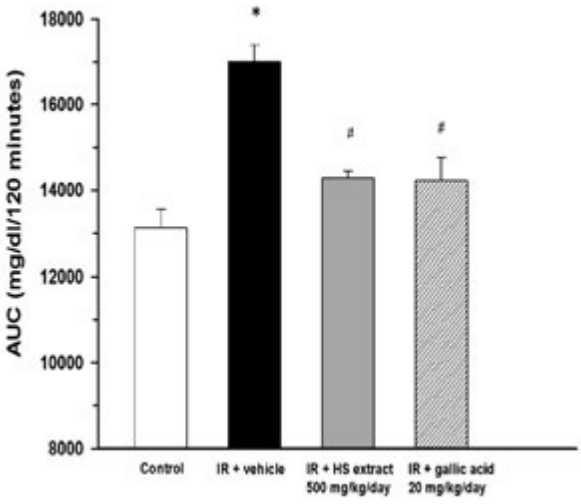

Figure 1 Effect of HS extracts and gallic acid on AUC of OGTT in insulin-resistant rats. * p < 0.05 vs. control, # p < 0.05 vs. IR + vehicle (n = 6/group)

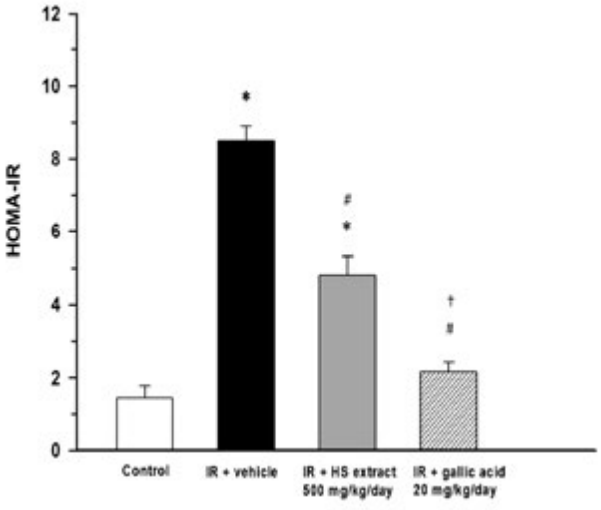

Figure 2 Effect of HS extracts and gallic acid on HOMA-IR score in insulin-resistant rats. * p < 0.05 vs. control, # p < 0.05 vs. IR + vehicle, † p < 0.05 vs. IR + HS extract 500 mg/kg (n = 7/group)

Effect of HS extract on oxidative stress status

Plasma MDA levels were increased in the IR rats (5.5 ± 0.7 μM) comparing to those of rats fed with a normal diet (2.5 ± 0.3 μM) (p<0.05). Interestingly, increasing of plasma MDA levels in IR rats was

attenuated by HS extract (3.6 ± 0.5 μM). In addition, a reduction of plasma MDA (3.3 ± 0.2) in IR rats treated with gallic acid for 4 weeks was also observed (Figure 3).

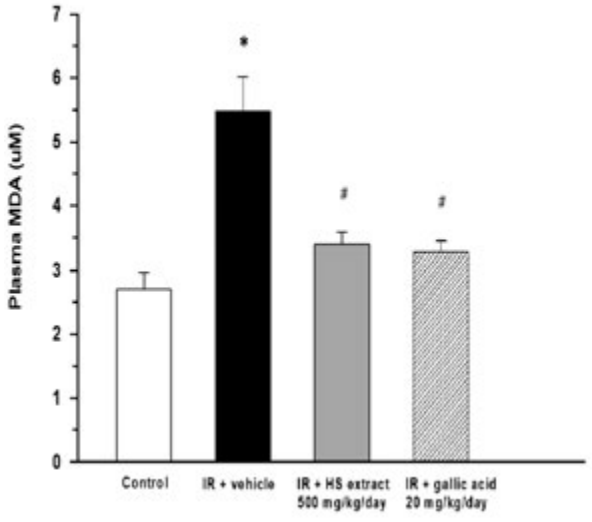

Figure 3 Effect of HS extracts and gallic acid on plasma MDA in insulin-resistant rats. * $p < 0.05$ vs. control, # $p < 0.05$ vs. IR + vehicle (n = 7/group)

5. Discussion

This present findings showed that feeding of HFD in rats for 14 weeks led to an insulin-resistant condition and an increase in oxidative stress. According to there was an increase in FBG levels, HOMA-IR score, an impairment of OGTT in rats received HFD. Furthermore, increased plasma MDA level was presented in this animal model. Administration of either HS extract or gallic acid evidently improved insulin resistant conditions, which was associated with an improvement of oxidative stress status by reducing lipid peroxidation.

We have found that HFD feeding caused the increase of FBG, HOMA-IR, and impaired OGTT, which indicated insulin-resistant status in this animal model. Previous study illustrated that daily intake of a HFD can develop insulin resistance in rats which related to increased FBG, impaired OGTT [14, 15]. The mechanisms of HFD induced insulin resistance remained unclearly; however, it may involve the reduction of the insulin-stimulated IRS-1 as well as PI3-kinase activity in liver and muscle cells [16].

Plasma MDA levels is a marker from lipid peroxidation that implied increase in oxidative stress. Increased lipid peroxidation and impaired antioxidant status have been found in HFD model [17]. Bell and coworkers (2000) found that there were increases in plasma glucose, insulin concentrations and oxidative stress in rats fed with diet high fructose for 12 weeks [18]. These observations were consistent with our results that HFD induced oxidative stress by increasing plasma MDA levels.

Our results presented that administration of HS extract reduced FBG levels, HOMA-IR score, as well as recovered OGTT in IR rats. These effects associated with decreased of oxidative stress status which was supported by the reduction in plasma MDA levels. The reduction of AUC from OGTT and HOMA-IR score indicated insulin resistance in a HFD feeding rats was ameliorated by HS extract treatment. Similarly, previously studies have described 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 [19]. It is well established that *Hibiscus sabdariffa* L. contains a variety of bioactive compounds with antioxidant properties, such as anthocyanins and phenolic compounds [20, 21]. Anthocyanins (including, cyanidin and delphinidin) have strong antioxidant activity in a liposomal system [22] and could ameliorate hyperglycemia and insulin sensitivity in diabetic mice [23].

This study we used gallic acid as positive control and the result showed that gallic acid reduced FBG levels, AUC of OGTT, HOMA-IR and plasma MDA in 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 [24]. Therefore we may imply that HS extract improved insulin sensitivity could be mediated by its antioxidant capacity.

6. Conclusions

The present study found that chronic feeding HFD produced insulin resistance as well as oxidative stress in rats. Treatment with HS extract improved insulin resistance accompanied with decreased lipid peroxidation. This finding supports the beneficial effect of *H. sabdariffa* as an antioxidant source that improve insulin sensitivity. In order to confirm the antioxidant activity may involve the improvement of insulin-resistant conditions in this animal model.

7. Acknowledgements

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