Antihyperglycemic and antihyperlipidemic effect of Uraria crinita water extract in diabetic mice induced by STZ and food

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Uraria crinita (UC) is a traditional edible plant and has been used as herbal medicine for a long history in China. There is no report on its anti-diabetic activity. In the present study we evaluated antihyperglycemic and antihyperlipidemic effect of U. crinita water extract (UCWE) in diabetic mice. Type II diabetes was induced in mice by injection of streptozotocin (STZ) in combination with high fat and protein food. After 3 weeks, oral administration of UCWE significantly decreased the fasting blood glucose in oral glucose tolerance test (OGTT), as well as significantly suppressed the increase of blood glucose after glucose challenge. Plasma concentration of triglyceride (TG) and free fatty acids (FFAs) were also decreased. Furthermore, UCWE increased the concentration of plasma insulin. Taken together, UCWE exhibited hypoglycemic and hypolipidemic activity, indicating a beneficial effect in diabetes treatment.

Key words: Uraria crinita water extract (UCWE), diabetes, oral glucose tolerance test (OGTT), TG, FFAs, insulin.

INTRODUCTION

Diabetes is a chronic and non-infectious disease associated with metabolism disorders of carbohydrate, fats and protein. It is estimated that more than 1% of the population is affected by diabetes. The numbers are growing large year by year.

Diabetes is clinically characterized by hyperglycemia and hyperlipidemia, which cause severe complications such as tumor, cardiovascular and cerebrovascular diseases. Currently used drugs include insulin, sulfonylureas and biguanides. However, their preventive effect is sometimes modest and often brings about side effects.

Herbal medicines appear to be a potent alternative way with less side effects. Several herbal medicines have been reported to exhibit hypoglycemic or hypolipidemic effect in diabetic mice, such as Morinda citrifolia (Kamiya et al., 2008) and Inula japonica (Shan et al., 2006).

Uraria crinita (UC), Leguminosae, is a traditional edible plant in Southern China. It has been used for the treatment of swelling, coldness, ulcer and stomachalgia (Liu et al., 1995), with results indicating possible anti-inflammatory activities. UC had been reported to be effective in inhibiting stress ulcers (Hsu and Liu, 1983). In some places, such as Guangdong province and Guangxi province in south China, U. crinita water decoction has been used as folk medicine to treat Levis diabetes. However, there is no experimental evidence regarding its
anti-diabetic activity. In the present study, we demonstrate that *U. crinita* water extract shows potent anti-hyperglycemic and anti-hyperlipidemic effect in diabetic mice.

**MATERIALS AND METHODS**

**Preparation of *Uuria crinita* (UC) extract**

Raw UC was purchased in the market of Guangdong, China and dried in the sunlight for three days. Dry UC (500 g) was added to 4 L distilled water, boiled for 2 h, cooled to room temperature and filtered through 100 mesh sieve. The filtrate was concentrated to 75 ml in rotary evaporator at 60°C. The obtained UC water extract was termed as UCWE and stored at 4°C.

**Reagents**

Kit for measuring blood glucose was purchased from Johnson and Johnson, USA. Kits for measuring blood insulin, triglyceride (TG) and free fatty acids (FFAs) were purchased from Wako Chemicals, USA. Streptozotocin (STZ) and metformin (Met) were purchased from Sigma-Aldrich, USA. The other chemicals were reagent grade from commercial source. High fat and protein diet contained about 18.3% carbohydrate, 30% lipid, 20% protein and 31.7% other ingredients.

**Animal studies**

Male C57BL/6J mice (5 - 6 weeks old, weighing 16 - 22 g) were purchased from Experimental Animal Center of Southern Medical University. Mice were acclimatized for 7 days under controlled conditions before the start of experiment: temperature, 20 - 25°C; humidity, 60 - 65%; 12 h light–dark cycle.

Diabetic mice were induced by intraperitoneal injection of streptozotocin (STZ, 100 mg/kg B.W.) in combination with high fat and protein food. The injection of STZ was given once a week successively for two weeks. Mice were fed with diet of high fat and protein from the beginning of injection. Two weeks later, blood samples were collected by tail nipping and the mice with blood glucose over 200 mg/dl (diabetic) were selected in the study. All mice were allowed free access to water and diet with high fat and protein during experiment.

Diabetic animals were randomly divided into 5 groups. Group I served as normal mice control and group II diabetic control. Both groups received distilled water only. Group III and IV were diabetic mice received UCWE (at the dosage equaling to 3 and 9 g/kg raw UC b.w., respectively). Group V was diabetic mice treated with standard drug metformin (MET, 500 mg/kg b.w.). The vehicle or test drugs were administered orally once a day successively for 21 days. At the end of the experiment, oral glucose tolerance test (OGTT) was performed and blood was collected from the eyes (venous pool) under aether anaesthesia to determine plasma levels of Insulin, TG and FFAs.

**Oral glucose tolerance test (OGTT)**

After overnight fasting, mice were administered orally with glucose solution (2 g/kg b.w.). Concentration of blood glucose was determined before the administration of glucose and 30, 60, 120 min later. Blood glucose concentration was measured following the commercial kit instructions.

**RESULTS**

**Effect of UCWE on glucose tolerance**

Results of glucose tolerance are shown in Table 1. Oral administration of UCWE decreased fasting blood glucose in comparison to diabetic mice. The most potent effect occurred at dose equaling to 9 g/kg (b.w.) raw UC, which is statistically significant (p < 0.05). After glucose challenge, blood glucose increased drastically in all mice. For normal mice, blood glucose returned to baseline at 120 min. While in all diabetic models, high blood glucose concentration sustained, indicating impaired glucose tolerance. UCWE significantly suppressed the increase of blood glucose at 30, 60 and 120 min in comparison to diabetic mice. The reduction percentage in control diabetic mice was 8.56 and 36.4% at 60 and 120 min. Whereas reduction percentage was 15.9 and 15.94% at 60 min and 46.67 and 49.1% at 120 min in mice receiving UCWE at dose equaling to 3 g/kg (b.w.) raw UC and 9 g/kg (b.w.) raw UC, respectively.

**Effects of UCWE on plasma insulin**

As shown in Figure 1, plasma Insulin decreased in control diabetic mice in comparison to normal mice. UCWE significantly increased plasma insulin level in diabetic mice in a dose-dependent manner (p < 0.05).

**Effects of UCWE on plasma TG**

Figure 2 shows the effect of UCWE on plasma TG. Plasma level of TG increased drastically in control diabetic mice in comparison to normal mice. Oral administration of UCWE led to significant decrease of TG in diabetic mice (p < 0.01).

**Effects of UCWE on plasma FFAs**

Effects of UCWE on plasma FFA is shown in Figure 3. Compared with normal mice, plasma levels of FFAs increased in control diabetic mice. UCWE was able to decrease FFAs in diabetic mice, with a statistically
significant effect (p < 0.01) at dose equaling to 3 g/kg raw UC.

**DISCUSSION**

Hyperglycemia is the most critical problem in diabetes. In the present study, UCWE exhibited potent anti-hyperglycemic effect. As shown in the results, UCWE significantly decreased fasting blood glucose, as well as significantly suppressed the increase of blood glucose after glucose challenge. Further, UCWE significantly increased plasma concentration of insulin, indicating that hyperglycemic effect was mediated by the enhanced secretion of insulin.

It is well understood that STZ-induced diabetes is linked to the production of reactive nitrogen species (RNS) and oxygen species (ROS), which damages DNA of pancreatic β cells, leading to cell necrosis and loss of insulin (Szkudelski, 2001). In the present study, high fat
Figure 2. Effect of UCWE on plasma TG. Normal mice were orally given with distilled water as control (Group I, n = 10). Diabetic mice were orally administered with water vehicle (Group II, n = 10), UCWE at a dosage equaling to 3 g/kg(b.w.) raw UC (Group III, n = 9), UCWE at a dosage equaling to 9 g/kg(b.w.) raw UC (Group IV, n = 9), or 500 mg/kg b.w. metformin (Group V, n = 10), respectively. The administration was given once a day for successive 21 days. At 22nd day, blood was collected from the eyes (venous pool) under aether anaesthesia and subjected to determination of TG concentration. Results are presented as means ± SE. UCWE significantly decreased plasma TG level. F value=15.321 (p<0.01). *p < 0.05, **p < 0.01 vs group I; #p < 0.05, ##p < 0.01 vs group II.

Figure 3. Effect of UCWE on plasma FFAs. Normal mice were orally given with distilled water as control (Group I, n=10). Diabetic mice were orally administered with water vehicle (Group II, n=10), UCWE at a dosage equaling to 3 g/kg (b.w.) raw UC (Group III, n=9), UCWE at a dosage equaling to 9 g/kg (b.w.) raw UC (Group IV, n=9), or 500 mg/kg b.w. metformin (Group V, n=10), respectively. The administration was given once a day for successive 21 days. At 22nd day, blood was collected from the eyes (venous pool) under aether anaesthesia and subjected to determination of FFAs concentration. Results are presented as means ± SE. UCWE was able to decrease plasma FFAs level, with statistically significant effect (p<0.01) at dosage equalling to 3 g/kg(b.w.) raw UC. F value=5.352 (p<0.01). *p < 0.05 vs. group I; ##p<0.01 vs.group II.
and high protein food may also cause increased oxidative stress in diabetes, resulting in further injury to pancreatic β cells. Recent pharmacognostic identification confirms that *U. crinita* water extract contains flavonoids, saponins and reducing sugar (Lin, 2008). Most plant derived flavonoids show antioxidant activity. In fact it has been shown that UC methanol extract showed potent antioxidant effect by decreasing production of RNS and ROS (Yen et al., 2001). Given that methanol is an organic compound with least polarity and is intermiscible with water, together with the confirmation of flavonoids in UCWE, we assume that UCWE exerts antioxidant activity. Increased plasma insulin by UCWE may be due to the reduction of oxygen radicals in diabetic mice, which facilitate the regeneration of pancreas β cells. The study on this hypothesis is being carried out in our lab.

It is accepted that lipoprotein disorders in diabetes are associated primarily with abnormalities in triglyceride metabolism. In the presence of hypertriglyceridemia, enhanced activity of cholesteryl ester transfer protein (CETP) may accelerate exchange of VLDL triglyceride for HDL cholesteryl esters (Hayek et al., 1993). The triglyceride in HDL is a substrate for plasma lipases, which converts HDL to a smaller particle and makes HDL more rapidly cleared from the plasma (Horowitz et al., 1993). In the present study, plasma TG was significantly decreased by UCWE, indicating a helpful effect in controlling dyslipidemia in diabetes.

The present study showed that UCWE was able to decrease plasma FFAs, with a significant reduction at dose equaling to 3 g/kg (b.w.) raw UC. There is a strong relationship between insulin resistance and circulating free fatty acids (FFAs). Elevated plasma FFAs induces skeletal muscle insulin resistance and liver insulin resistance (Delarue et al., 2007). Besides, increasing oxidative stress may also contribute to the induction of insulin resistance. Whether UCWE could attenuate insulin resistance caused by FFAs or oxidative stress needs to be further confirmed.

Taken together, the present study is the first report on the anti-diabetic activity of UCWE. *U. crinita* is a traditional edible plant in Southern China. Our results indicate that *U. crinita* can be exploited as a supplement in diabetes treatment.

REFERENCES


