Betanidin significantly reduces blood glucose levels in BALB/c mice fed with an atherogenic diet

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Abstract: Six weeks BALB/c mice were fed with an atherogenic diet for 24 weeks and purified water ad libitum. An experimental group was given betanidin, orally, during the last 40 days of the experiment at a dose of 9.6 mg per mouse per day. Negative controls were fed with standard rodent chow only. Glycemia was measured at the end of the experiment, after overnight fasting. The group treated with betanidin presented a highly significant reduction of 50.94% compared to positive controls. We conclude that betanidin reduces glycemia in BALB/c mice by an unidentified mechanism.

Keywords: betalains, betanidin, blood glucose, hypoglycemicant

Introduction

Betalains are hydrophilic nitrogenous pigments found in most plants from the order Caryophyllales. These pigments are mainly found in the vacuoles of all their tissues1. Betalains confer these plants their yellow, orange, red and violet colours2 and are powerful antioxidants3,4. In the course of evaluating the antioxidant properties of betanidin5, the aglycone of the most common betain, in the pathophysiology of atherosclerosis, we found that betanidin has an effect in the blood glucose levels in BALB/c mice fed with an atherogenic diet.

Results and Discussion

Betanidin treatment produced a 50.94% reduction in the glycemia of mice as compared to the mean from the positive control group (Table 1). This reduction was highly significant (p < 0.00001) compared to both control groups (Figure 1). There were not significant differences between positive and negative controls.

The hypoglycemic effect observed for betanidin we describe here has not been reported before6. There are also not reports of any effect of betalains in the insulin or glucose tolerance. As with other betalains7,8–9, the main biological action of betanidin known to date in animal biomolecules is its antioxidant capacity10. Betalains have also been shown to induce the expression of quinone reductases11, to inhibit the expression of ICAM-112 and to decrease the DNA methyl transferase activity13. However, none of the effects mentioned can directly explain the hypoglycemic effect observed. Unfortunately, we could not measure glucose or insulin tolerance or levels of expression of the second; therefore, it is not possible to determine if these two factors suffer changes with the administration of betanidin. Moreover, we can only determine that betanidin produce a hypoglycemic effect but it is not possible with the available information to determine if it has also an anti-hyperglycemic effect. We did not quantify the hypoglycemic effect with different doses of betanidin nor the presence of this effect in other betalains. In respect to the latter, since the antioxidant capacity and the other effects mentioned before are considered to be present in all betalains3,4,6–8,10–12,14,15, it may be possible that the hypoglycemic effect is present in all this group of molecules; further studies will determine if this is the case.

Experimental Section

Animals. Six weeks inbred BALB/c mice (Harlan Laboratories, Indiana, USA) were randomly assigned into cages (n = 5 per cage), acclimatized for 2 weeks in a 12 hours...
At the start of the experiment, the protocol. The findings here
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from the University of Colima and conducted in accordance
betanidin was given orally to the betanidin group at a dose of 9.6 mg per mouse per day. After an overnight fasting (water was not suspended), decapitation was performed as part of a parallel experimental protocol. The findings here described resulted as parallel data from a bigger research project evaluating the effect of betanidin in dyslipidemia. All animal procedures were approved by the Bioethics Committee from the University of Colima and conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

**Betanin Obtainment.** Betanin extraction and purification
Fresh red-purple pitaya fruits (*Hylocereus ocamponis*) were purchased from a commercial plantation in Jalisco (Mexico). Upon arrival of fruits, they were washed and peeled by hand. Skins were discarded and the fruit flesh was macerated in a blender until being completely homogenized. For pigment extraction, 1 part of this sample was shaken with 2 parts of solvent for 5 minutes and allowed to stand for 15 min; solvents used were 80% acetone, 80% methanol and ultra purified water. After, the mixture was filtrated through a nylon cloth and centrifuged at 3500 rpm for 15 min. Afterwards, the supernatant was filtered through YM-10 membranes (Millipore) to remove proteins, and the filtrate was used for pigment analysis and further purification. All was performed at room temperature. Betanin was purified by FPLC and confirmed by HPLC as performed by Gandía-Herrero, *et al*.

**Betanin synthesis** An enzymatic hydrolysis of purified betanin with β-glucosidase was performed as stated by Gandia-Herrero, *et al*. Transformation was complete according to HPLC analysis.

**Blood Glucose Levels.** A OneTouch Ultra test strip (LifeScan, California, USA) was filled with a drop of blood immediately after euthanasia. Blood glucose levels were measured using a OneTouch UltraMini meter (LifeScan, California, USA) and the results were expressed in mg/dl. Calculations and graphs were carried out in the Microsoft Excel software (Microsoft Corporation, Washington, USA).

**Statistical Analysis.** Data represent mean ±SEM. One-way ANOVA test was used as a measure of significance. Differences with p values of less than 0.05 were considered statistically significant. Statistical analyses were carried out in the Microsoft Excel software (Microsoft Corporation, Washington, USA).

**Figure 1.** Effect of betanidin in blood glucose levels. Oral administration of betanidin produced a significant reduction of around 50% in the blood glucose levels in BALB/c mice. * = p < 0.00001

Electronic Supplementary Material
Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s13659-012-0034-z and is accessible for authorized users.

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