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RESEARCH ARTICLE

The effect of flabelliferins of palmyrah fruit pulp on intestinal glucose uptake in mice

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Abstract: Palmyrah (*Boarssus flabellifer* L.) is a tree found growing in arid climates. It has an edible fruit pulp. Previous work had shown that Institute of Cancer Research (ICR) mice fed with 10% fruit pulp in standard feed showed reduced weight gain due to bitter steroidal saponins termed flabelliferin-II. The effect of this pulp and flabelliferin II on glucose uptake was studied. At a dose of 10mg/mouse, mixed flabelliferins (with 2.5 mg flabelliferin II) reduced blood glucose after glucose challenge ($p < 0.001$), increased faecal glucose ($p < 0.001$) and intestinal glucose ($p < 0.001$), but not faecal glucose ($p = 0.62$). The latter is probably due to the removal of flabelliferin triglycoside which is antimicrobial. The antimicrobial component and two other flabelliferins did not show the above effect. As the pulp is non-toxic it may find application as a functional food.

Keywords: Flabelliferins, glucose uptake, ICR mice, palmyrah fruit pulp.

INTRODUCTION

Flabelliferins, a family of steroidal saponins in the fruit pulp of palmyrah (*Borassus flabellifer* L., Family: *Arecaceae*) were first highlighted in 1994[1]. Of the flabelliferins isolated so far from the crude flabelliferin mixture, a tetraglycoside flabelliferin-II (F-II), with two rhamnosyl moieties and two glucosyl moieties, is responsible for the bitterness in palmyrah fruit pulp.[1] F-II has also been shown to inhibit ATPase of ghost red blood cells.[2] Debittering of Palmyrah Fruit Pulp (PFP) by the enzyme naringinase causes the loss of F-II (and bitterness) and another flabelliferin, flabelliferin B (FB), a branched flabelliferin triglycoside.[3] Flabelliferin B was shown to have potent anti-yeast and anti-bacterial activity.[4] It had been observed that PFP caused weight loss in Institute of Cancer Research mice (ICR) despite there being no change in isocaloric feed intake.[5] Feeding of non-bitter PFP (no F-II) did not result in such weight loss thus indirectly implicating F-II as the causative agent for the observed weight loss. Flabelliferins are naturally associated with a uv-active binder[6] which could have an effect on the extent of their activity.

The objectives of this study were to determine: (i) whether PFP induced weight loss[5,6] were reproducible, (ii) the cause for the weight loss, (iii) whether separation of the uv- active binder would affect bioactivity (iv) and whether therapeutic applications would be feasible.

METHODS AND MATERIALS

Biological materials

Experimental animals : Inbred, homogeneous ICR mice (4 wk old, 20±5 g body weight, weanling males for weight gain studies, and 6 wk old, 35±5 g body weight males for other studies) were purchased from the Medical Research Institute, and the experiments were conducted at the animal house, University of Sri Jayewardenepura. The mice were maintained in a temperature-controlled room (30 °C) under 12 h light/dark cycle (dark phase 6 p.m to 6 a.m). They were fed with the rat and mouse breeding feed recommended by the World Health Organization (WHO)[7] or 10% PFP containing pellet feed and water *ad libitum*. Feed intake and weight gain were determined by methods previously described.[3,5]

Plant materials: Fruits of palmyrah were collected from Kalpitiya in the North-West of Sri Lanka. The fruit pulp was extracted from its fibrous matrix by extracting manually with a spoon, using water in the ratio of 2:1.[4]

Extraction of flabelliferin mixture from PFP: The flabelliferin mixture was extracted using methanol followed by de-carotenising with petroleum ether (60-80 °C).[1] The sugar was separated from extracts using a dry cellulose column.[1]

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Separation of flabelliferins: Separation of flabelliferins was conducted by using a chromatotron[8] (Harrison, California, USA). When necessary, further purification was carried out using preparative thin layer chromatography (300 mm silica gel G60 using butanol: ethanol; ammonium hydroxide, specific gravity 0.88 = 7:3:4). Spraying with anisaldehyde reagent was necessary to monitor fractions and test them for purity.[1] All flabelliferins contain a uv-active binder.[6] This was separated using a mixture of iso-propanol:methanol (1:1) in a chromatotron to obtain pure flabelliferins.[8]

Assay of flabelliferins: This was conducted using a computerized densitometer by methods described previously.[8]

Administration of 10% PFP containing feed: PFP containing feed was prepared by substituting one-third the maize with 10% PFP on dry basis (when PFP was used) in WHO standard rat and mouse breeding feed. Test and control diets were isocaloric. Mice (36) were divided into 6 groups (6 in each), 3 control groups and 3 test groups.

(I) One set (a control and a test group) was used for the weight gain study and at the end of 2 wks their fasting blood sugar levels were tested.

(II) In the 2nd set, 10% PFP containing feed was given to the test group and the WHO standard rat and mouse breeding feed was given to the control group. Faeces were collected on the following day for testing of reducing substances.

(III) The remaining set of animals were given 10% PFP containing feed (test) and WHO standard rat and mouse breeding feed (control) for a day. On the following day they were given a glucose load and after 1.5 h they were sacrificed to collect blood and intestinal samples.

Administration of flabelliferins: The dosage of mixed flabelliferins administered to the test group was 10 mg/ 50 g mouse in 0.5 mL distilled water (calculated on the basis of the yield of flabelliferins after separation from PFP and food intake of a mouse). Controls were given the same volume of water. The dosage of mixed flabelliferins without ultra violet (uv) binder administered was 2 mg/ 50 g mouse in 0.5 ml distilled water.

The dosage of purified flabelliferins (calculated on the basis of preliminary experiment) was: F-II, 1 mg/50 g mouse, FB, 3 mg/50 g mouse, FD+FE, 1.5 mg/50 g mouse. Extractives were administered orally using a Sondi needle by the gastric garage method.

Glucose challenge: Mice were fasted overnight and glucose (Glaxo -Welcome Co.,) dissolved in distilled water, was orally administered to mice at a dose of 1.5 g/kg body weight in a volume of 0.5 mL.

Collection and assay of faeces: Faeces were collected 24 h after administration of glucose and the total sugar content was determined by the Dinitrosalicylic acid (DNS) method.[9]

Estimation of glucose in blood

Fasting blood glucose: Animals were fasted for 12 h and they were sacrificed under diethyl ether anesthesia. Blood (0.5 mL) was drawn by cardiac puncture and the blood glucose concentrations analysed immediately using a reagent kit (DMA, USA) employing the glucose oxidase method.[10]

Post prandial blood glucose: Animals were sacrificed under diethyl ether anasthesia, 90 min post glucose loading (2 h after administration of extract) and the glucose level was determined as before.

Collection of intestinal samples and determination of glucose content in the intestinal wash [11]: Animals were fasted overnight and the test group was orally administered the extractive 30 min prior to the glucose challenge. The control group was orally administered distilled water (0.5 mL/50 g mouse) at the same time. The animals in the test group and the control group were sacrificed 1.5 h after the administration of glucose load under diethyl ether anasthesia and their intestines removed. Intestines from each animal were washed with 25 mL of distilled water and the washings centrifuged at 3000 rpm for 10 min. The supernatant was tested by the glucose oxidase method[10] to determine the glucose content.

Statistical analysis

All the results are presented as (mean ± S.E.) Statistical analysis was carried out in Microsoft Excel. The significance was tested by Student's t-test. A probability level of $p < 0.05$ was chosen as the cut off point of statistical significance, but accurate p values were calculated using the computer software.

RESULTS

1. Flabelliferin profile. Total flabelliferin content in the PFP sample used in the present study was 0.5%/ PFP

(50 mg/100 g). The sample was a bulk sample from Kalpitiya, one of the main sources of PFP. The percentages of the pure flabelliferins (per total flabelliferins) were F-II – 51%, FB –3.9%, FD+FE –41.5% in this sample.

2. Studies using 10% PFP.

(i) Animal set 1. Weanling ICR mice fed over a period of 2 weeks with 10% PFP containing feed showed a marked decrease in weight gain ($p=0.007$, Table 1) compared to control mice fed with the PFP free diet, although food intake did not decrease significantly ($p=0.7$). This had been previously reported[5,6]. No significant difference was found in the fasting blood sugar levels between the test and control animals ($p=0.64$).

(ii) Animal set 2. The reducing sugar content in the faeces of the test group of animals was significantly higher ($p<0.0001$) than in the control group, while the faecal fat content was similar (14.77% in test group and 14.47% in controls).

(iii) Animal set 3. The test group of animals had significantly lower blood glucose levels ($p<0.001$) with no significant changes in the intestinal glucose content ($p=0.21$) after a glucose challenge (Table 1).

3. Studies using mixed flabelliferins.

Animal set 1. Test mice ($n=6$) were administered 10 mg/50 g mouse mixed flabelliferins in 0.5 mL of water for 7 days. Controls were given the same volume of water. At the end of the 7 day period of feeding with the mixed flabelliferins, the test animals had a lower weight gain than ($p=0.01$) the control animals. However, fasting blood sugar did not vary markedly ($p=0.11$).

Animal set 2. There was no significant difference between faecal weight of test animals and controls. However, after a glucose challenge, faecal sugar ($p<0.01$) and intestinal glucose ($p<0.001$) in the test animals increased significantly while blood glucose showed a marked decline ($p<0.0001$) when compared to the corresponding values in control animals (Table 2). Separating the fluorescent binder from mixed flabelliferins gave larger decline even when the dose was reduced to 2 mg/50 g mouse(Table 2).

4. Separated flabelliferins.

F-II. Oral administration of the flabelliferin F-II (MW = 1030) containing uv binder (MW = 544) after a glucose challenge resulted in a significant increase in intestinal glucose content ($p< 0.001$) and decrease in blood glucose level ($p<0.001$) with no change in faecal sugar and faecal weight, despite the dose being only 1 mg/50 g mouse (Table 2).

FB and FD+FE. Administration of FB (3 mg/50 g mouse) and FD+FE (1.5 mg/50g mouse) resulted in a reduction in intestinal glucose content and elevation in blood glucose level after a glucose challenge. This was the reverse of the effect observed with F-II (Table 2).

Table 1: Effect of 10% PFP containing feed on weight gain and blood and intestinal glucose after a glucose load (1.5 g/Kg BW)

	Control	Test	p value
A. Average weight gain in 2 weeks	12.2±0.56	6.9±1.48	0.007
B. Mean blood glucose level (mg/dL)	185.3±5.7	162.9±3.5	0.001
C. Mean glucose of intestinal wash	9.7±1.6	10.8±3.7	0.21

A: test animals = 6, were given 10% PFP containing feed and the control group was given WHO standard rat and mouse breeding feed for two weeks.

B and C: after feeding for one day the mice were given a glucose load and after 1.5 h they were sacrificed to collect blood and intestinal samples.

Mean ±S.E.

Table 2: Effects of flabelliferins on glucose content in blood, faeces and intestinal wash after glucose challenge.

	Mean blood glucose mg/dL	Mean faecal sugar content mg/dL	Mean glucose in intestinal wash mg/dL
A. Mixed flabelliferins (10 mg/mouse) containing uv binder			
Control	181.8±16.1	506±59.7	21.7±0.8
Test	102.9±7.9	720±28.1	126.3±3.0P
P value	< 0.0001	<0.01	<0.001
B. Mixed flabelliferins (2 mg/mouse) without uv binder			
Control	297.5±4.3	Not tested	32.8±2.3
Test	178.6±5.1	Not tested	72.2±2.1
P value	< 0.001		< 0.001
C. Separated flabelliferins (1mg F II, 3mg FB, 1.5mg FD+FE with uv binder/mouse respectively)			
Control	110±1.6	967±6.2	17.2±0.8
Test (F-II)	62.6±3.6	960±13.9	345±1.1
P value	<0.001	=0.62	<0.001
Control	195.6±1.0	Not tested	48.8±1.9
Test (FB)	197.9±1.1	Not tested	44.0±1.8
P value	=0.14		=0.1
Test (FD+FE)	190.3±2.7	Not tested	40.9±2.6
P value	=0.09		=0.07

Test and control mice (n=6 each) were fasted for 12 h, and a glucose load (1.5 g/Kg BW) was given 30 min after administration of test flabelliferins. Glucose content in blood and intestinal wash was determined 2 h after extractive following sacrificing with diethyl ether anesthesia. Faeces were collected in a separate trial, 24 h after administration of F-II, and analysed for sugar in test and controls. A, B, and C were separate trials with their own controls. C had two controls, one for F-II trial and another for the other flabelliferins.

Mean ±SEM

DISCUSSION

The results confirm previous observations[3,5] that administration of PFP containing feed results in a significant weight loss. The insignificant effect on fasting blood sugar of mice fed on 10% PFP could probably be attributed to the high sugar content (about 80% on a dry weight basis of PFP) which may serve to counteract the hypoglycaemic effects of the active principle. In addition, gluconeogenesis that would occur during fasting may also contribute to maintaining blood glucose homeostasis.

Experiments with separated flabelliferins, clearly showed that F-II (flabelliferin tetraglycoside) is the compound that inhibits absorption of glucose from the intestinal lumen and this results in a reduction of blood glucose on glucose challenge (an anti hyperglycaemic effect) and probably leads to the reduced weight gain observed previously[3,5]. It is interesting that faecal glucose content was not found to be significantly higher on administration of pure F-II. This could be attributed to absence of the antimicrobial flabelliferin (FB)[4] in pure F-II. FB, that is present in mixed crude flabelliferins is known to inhibit a wide range of bacteria[4], possibly inhibits bacteria of the large intestine and leads to high glucose in the faeces of mice administered with crude flabelliferins or fed with 10% PFP containing feed. The effects of flabelliferins plus and minus the uv active binder on glucose challenge show that separation of the binder increases the efficiency with which the flabelliferins, probably flabelliferin F-II, reduces intestinal glucose uptake even when calculations are made on molar basis. It should be noted that the F-II constitutes 50% of the mixed flabelliferins while flabelliferins FB, FD and FE make up most of the rest. This study shows that FB, FD and FE produce no decline of glucose uptake.

The overall results have implications on the use of F-II as either an anti-obesity or anti-diabetic food component. The fact that PFP is non-toxic to mice even at 50% incorporation in feed[12] lends weight to this possible application.

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