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An investigation of palmyrah fruit pulp mediated inhibition of intestinal glucose uptake and its toxicity

By

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Thesis submitted to the University of Sri Jayewardenapura for the award of the Degree of Doctor of Philosophy in Biochemistry on 'An investigation of palmyrah fruit pulp-mediated inhibition of intestinal glucose uptake and its toxicity' on June 2005

DECLARATION BY CANDIDATE

The work described in this thesis, was carried out by me, under the supervision of Prof. E. R. Jansz (Dept. of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Prof. M. I. Thabrew (Dept. of Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Kelaniya) and a report on this has not been submitted in whole or in part to any University for another Degree/Diploma.

09 - 01 - 2006
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Date

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Signature of candidate

DECLARATION BY SUPERVISORS

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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Prof. E. R. Jansz

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Prof. M. I. Thabrew

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ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine transaminase
AOAC	Association of Official Analytical Chemists
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BAW	n- butanol : glacial acetic acid : water
BEN	n- butanol : ethanol : ammonia
DM	Diabetes mellitus
FAB/MS	Fast Atom Bombardment- Mass spectrometry
FBS	Fasting blood sugar
F-I	Flabelliferin tetraglucoside
F-II	Bitter flabelliferin tetraglycoside
F_B	Anti bacterial flabelliferin triglycoside
F_C	Inactive flabelliferin triglycoside
F_D	Inactive flabelliferin diglycoside
F_E	New flabelliferin
F_F	New flabelliferin
F_M	New flabelliferin
F_N	New flabelliferin
γ-GT	Gamma glutamyl transpeptidase
GC	Gas Chromatography
Glc	Glucose
Hb	Haemoglobin concentration
H and E	Haematoxylin and eosin
HPLC	High Performance Liquid Chromatography
IDDM	Insulin dependent diabetes mellitus
MPLC	Medium pressure Liquid Chromatography
MW	Molecular Weight
NIDDM	Non insulin dependent diabetes mellitus
PCV	Packed Cell Volume

PFP	Palmyrah fruit pulp
PPBS	Post prandial blood sugar
^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectrometry
RBC	Red blood cell count
Rha	Rhamnose
TLC	Thin Layer Chromatography
WBC	White blood cell count

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ABSTRACT

Reduced weight gain by Institute of Cancer Research (ICR) mice had been previously reported after feeding 10% palmyrah (*Borassus flabellifer*) fruit pulp (PFP) containing feed. In those studies F-II, a steroidal tetraglycoside of β-sitosterol (MW, 1030) was strongly implicated as the active principle. The objective of this study was to confirm the above, its effect on glucose uptake and determine the mechanism of action and some of the factors affecting this effect.

The study has provided evidence that confirms the weight reducing property of PFP and also demonstrated its ability to inhibit intestinal glucose uptake in mice. Studies showed that feeding of PFP had no effect on fasting blood sugar levels of mice ($p=0.64$) but significantly reduced the liver glycogen content of test mice ($p<0.01$, 11%). A mixture of flabelliferins (dose: 10mg/50g mouse) extracted by the methods described previously on administration to mice after a glucose challenge resulted in a decline in serum glucose levels ($p< 0.0001$, 43%), increased intestinal glucose levels ($p<10^{-10}$, 83%) and increased faecal glucose levels ($p<0.01$, 29%). On isolating F-II and other flabelliferins (F_B, F_D/F_E) it was found that only F-II has the ability to lower serum glucose ($p<0.001$, 43%) and increase intestinal glucose ($p<0.001$, 50%). This effect was shown at a dose of 1mg F-II/50g mouse. The findings of the mice study were supported by evidence from a study conducted with mild diabetic type-II patients. In the human study it was decided to use pinattu, a dried PFP which can be classified as fruit leather. The dose of 6g of pinattu (30 mg F-II) was calculated on the basis of IC_{50}

studies and mean human body weight. When pinattu was given to mild, type-II diabetic patients who are not under any therapeutic regimen there was a reduction of 24-48% serum glucose after a glucose challenge (post prandial) when compared with their control (post prandial) values. It has also been shown that the inhibition of intestinal glucose uptake by PFP is mediated mainly by F-II and not by the fibre it contains. A fibre control used in the human study showed that a small part of this is due to fibres (2-11% reduction in serum glucose level) present in PFP.

It has been known for some time that all flabelliferins are bound by a UV active binder. The present study revealed that this binder was comprised of a major carotenoid component identified as phytofluene and a minor component phytoene. Removal of the binder increased the efficiency with which F-II inhibited intestinal glucose uptake even when the dose was reduced to 2 mg mixed flabelliferin/50g mouse.

Inhibition of Na^+/K^+ ATPase activity was found to be a mechanism by which F-II mediates its inhibitory action on intestinal glucose uptake (the IC_{50} values were 9×10^{-5}M and 5×10^{-5} M with and without UV binder respectively). Computational studies showed that the lowering of the inhibition by the binder was probably due to the distortion of the carbohydrate moiety of the F-II, which is strongly connected to the activity the molecule. The beneficial effects of any pharmaceutical agent depend on it being non toxic. Results showed that feeding 10% PFP containing feed for one week (short term) or feeding 10% and 50% PFP containing feed for 30 days (long term) did not show any increase in the levels of key hepatic enzymes *viz;* alkaline phosphatase, alanine transaminase and aspartate transaminase in the test group when compared with that of the controls. Haematological parameters tested (haemoglobin level, packed cell volume, red cell count and white cell count) were not significantly different in the test

and control groups. Creatinine levels in the test and control groups were not significantly different from each other indicating no renal damage. Microscopic examination of liver, kidney and intestine of test group did not show any pathological changes when compared to those in the control group.

From the overall results it may be concluded that PFP possibly has an application in treating type –II diabetics most probably those that are obese. However the dosage of PFP pinattu must be closely monitored as different PFPs contain different amount of F-II.