



A pilot study on palmyrah pinattu (dried fruit pulp) as an anti-diabetic food component

Inoka ULUWADUGE^{1,2*}, Antoinette PERERA¹, Errol JANSZ¹ and Ira THABREW²

¹ University of Sri Jayewardenepura, Gangodawille, Nugegoda, Sri Lanka.

² University of Kelaniya, Ragama, Sri Lanka.

* Corresponding author, E mail: deepthi_uluwaduge@yahoo.com

ABSTRACT

The fruit pulp of palmyrah (*Borassus flabellifer* L.) has been shown to inhibit intestinal glucose uptake in mice, the active principle being a steroidal saponin, flabelliferin-II which inhibits intestinal ATPase in mice at 5×10^{-5} M level. Palmyrah fruit pulp (PFP) is widely used to manufacture many food products including dried PFP (pinattu), which has been consumed in North-East Sri Lanka for centuries. The present study was carried out to investigate whether PFP in the form of pinattu could reduce serum glucose levels of mild diabetic (Type-II) patients who were not on a drug regimen with a view to developing pinattu as an anti-diabetic food component. Patients (newly diagnosed, Type-II, mild diabetic patients) attending the diabetic clinic at the Family Practice Centre, University of Sri Jayewardenepura, Sri Lanka, were subjected to a glucose challenge (75 g/50 kg BW) after a 10 hour overnight fast and the blood glucose levels determined. On subsequent visits of each patient (3 days after the first visit) blood glucose was determined after administration of PFP in the form of pinattu (6 g/50 kg BW) or fibre (4 g/50 kg BW) extracted from PFP prior to the glucose challenge. The methodology employed was the cross over method where each patient was its own control. In all mild diabetic patients treated with pinattu, there was a significant reduction ($p < 0.01$, by 15-48%) in blood glucose concentration after a glucose challenge. Therefore the results of the present study suggest that pinattu (dried PFP) could be used as an anti-hyperglycemic agent.

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Key words: *Borassus flabellifer*, flabelliferin, palmyrah fruit pulp, blood glucose, diabetes.

INTRODUCTION

Palmyrah (*Borassus flabellifer* L, Family: *Arecaceae*) is present in the arid zone of India, Sri Lanka South and East Asia and African countries (Thabrew and Jansz, 2004). The fruit of this dioecious palm contains a yellow-orange pulp (PFP) covering 1-3 seeds. The pulp contains pectin, sugar, carotenoids and numerous steroidal saponins (flabelliferins) (Thabrew and Jansz, 2004). One of these flabelliferins, a tetraglycoside of β -sitosterol (Ariyasena, 2002) termed F-II (Nikawala, 2000) was suspected to reduce weight gain in ICR mice (Ariyasena et al., 2002a). This reduced weight gain is reversed when the pulp is treated with the enzyme naringinase, which hydrolyses the bitter F-II and another flabelliferin- FB (triglycoside).

Feeding non-bitter PFP to mice (with FB but no F-II) caused no reduction in weight gain (Ariyasena et al., 2002b). These findings suggested that F-II has an effect on growth of mice.

More recent studies (Uluwaduge et al., 2005a) with mice have demonstrated that F-II (1 mg/mouse) extracted from PFP can significantly inhibit the expected increase in blood glucose after a glucose challenge, while enhancing the level of faecal glucose, thus suggesting an inhibition of intestinal glucose uptake (Uluwaduge et al., 2005a). The results of this study also indicated that inhibition of Na^+/K^+ ATPase activity could be a mechanism by which F-II mediates its inhibitory action on intestinal glucose uptake in mice.

Previous investigations carried out by the authors have also demonstrated 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 result in any significant toxic side effects (Uluwaduge et al., 2005b).

Pinattu is a dried PFP, which has been eaten by people of the North-East of Sri Lanka for centuries; it is a pleasant flavored solid with a chewing gum like texture.

Since PFP has been demonstrated to be relatively non-toxic, the present study was undertaken to investigate whether PFP (in the form of pinattu) can be used as an anti-diabetic food component.

MATERIALS AND METHODS

Preparation of pinattu (Nikawala, 2000)

PFP (600g) was dried in layers in a 11/2m x 21/2m Mitchell forced draft tray drier (Mitchell, USA) at 60 °C using one tray for about 40 hours in Industrial Technology Institute (ITI), Colombo, Sri Lanka. This was prepared under strict supervision and under hygienic conditions (no microbial and chemical contamination). Finally dried layers of PFP (pinattu) were cut into pieces (6 g each) and packed and sealed in polythene and stored at -5 °C.

Extraction of fibre from PFP

Dietary fibre was isolated from PFP (600 g) by an ethanol precipitation using a modification (omitting incineration) of the methods described by Asp et al. (1983).

Extraction of bitter flabelliferin (F-II) from PFP

F-II was extracted and purified by the method described previously by Jansz et al. (1994).

Selection of human diabetic subjects

Characteristics of patients selected for the study are shown in Table 1. Patients (n=20, 8 male and 12 females, 45-60 years old and 45-55 kg BW) who attended the Family Practice Centre, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka and were confirmed as type-II mild diabetics, and who consented to participate in the study were invited to enter the study. Since mild diabetics, may vary in their response to a glucose challenge, the

cross-over method where each patient is its own control, was employed in this study.

Only those patients who were shown at their first visit to the clinic, to have fasting blood glucose level between 110- 200 mg/dl (mild, Type II diabetic or impaired glucose tolerance patients) and were not on any drug regimen were selected for the study. Ethical clearance for this study was obtained (No: 180) from the Ethics Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.

Glucose challenge, administration of pinattu and fibre to patients

Glucose challenge

Patients on the first visit to the diabetic clinic after 10 h fast were orally administered a dose of glucose (75 g of glucose dissolved in 300 ml of water). One hour and thirty minutes after administration of the glucose, a sample of blood (0.5 ml) was drawn by venepuncture for the determination of postprandial blood sugar (PPBS) by the glucose oxidase method (Hugget and Nixon, 1957). This was considered to be the control blood glucose value of each patient.

Administration of pinattu

Based on IC50 of intestinal ATPase by F-II (Uluwaduge et al., 2006), a dose of 6 g pinattu was selected for administration to diabetic patients. Three days after the 1st visit, each patient (fasted for 10 hours) was given 6 g of pinattu 30 minutes prior to the glucose challenge. Blood was drawn (1.5 hours after the glucose load) for the determination of blood glucose as before. This was taken as the test sample.

Administration of fibre

Three days after the test samples were taken, each patient (10 hour fasting) was orally fed with a suspension of PFP fibre (equivalent to 6 g of pinattu, 4 g fibre/100 ml water), 30 min prior to the glucose load and blood was drawn for the determination of blood glucose as before. This was taken as the fibre control.

Statistical analysis

The difference between the postprandial blood glucose levels after each meal was compared using one way ANOVA. The differences between groups were determined using post hoc S-N-K test.

RESULTS

The mean reduction in the blood glucose level of each subject brought about by pinattu (6 g) or PFP fibre (4 g) after a glucose challenge is summarized in Table 2. The reduction of blood glucose mediated by pinattu or fibre in each mild diabetic patient included in the study is shown in Figure 1. The pinattu used in the study, contained 50 mg F-II per 6 g.

Statistical analysis showed that there was a significant difference between the postprandial blood glucose level at base line and after ingestion of pinattu ($F=11.9$, $p<0.001$). There was no difference between the postprandial blood glucose levels at base line and after ingestion of fibre extracted from PFP.

Table 1: Characteristics of patients selected for the study.

Characteristic	Description
(a) Gender	Male-8 Female-12
(b) Age range	45-65 years
(c) Weight range	45-55 kg
(d) Fasting blood glucose level	110-200 mg/dl
(e) Any other systemic illness that needed treatment	No
(f) On anti-diabetic treatment at time of recruitment to the study	No

Table 2: Postprandial blood glucose level (PPBS) in type II diabetic patients (n=20) with or without treatment of pinattu or PFP fibre.

PPBS value	Mean PPBS (mg/dl)	Mean percentage reduction of in comparison with baseline
No treatment (base line)	281.3±92	-
Pinattu (6 g)	191±51.6	28.6*
Fibre (4 g)	250.1±56.4	5.9

Mean±SEM; * Significantly different from the base line value ($p<0.01$)

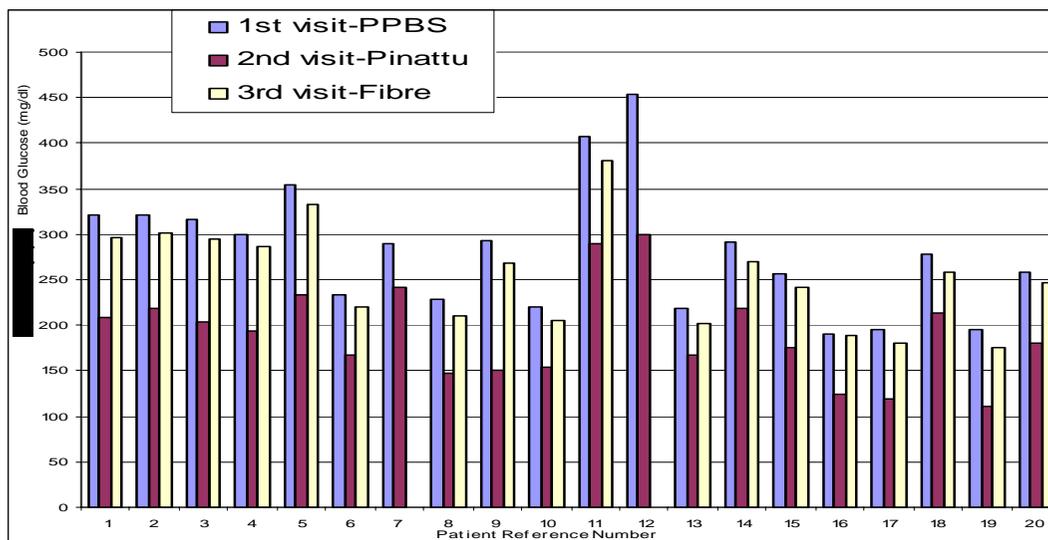


Figure 1: Postprandial blood glucose level (mg/dl) of each patient with or without treatment of pinattu and PFP fibre with respect to control values in individual patients (n=20). Patient No. 7 and 12 did not report for crude fibre trial.

DISCUSSION

Pinattu, a product prepared by drying PFP has been consumed by people of the North-East Sri Lanka for centuries with no documented evidence of toxicity (Thabrew and Jansz, 2004).

The present study was carried out to investigate whether PFP in the form of pinattu could reduce serum glucose levels of mild diabetic (Type-II) patients with a view to developing pinattu as an anti-diabetic food component. Results of the present study demonstrated that in mild, type-II diabetic patients administered with pinattu (6 g), there was a 15-48% reduction ($p < 0.05$) in the blood glucose after a glucose challenge when compared with their own control values. These findings suggest that pinattu can help in the reduction of postprandial blood glucose levels of mild diabetic, type-II patients who are not under any other drug regimen.

Previous study by Uluwaduge et al. (2005a) showed that fresh PFP can reduce the postprandial blood glucose level of mice. The overall results of this study together with the findings of the present investigation provide confirmatory evidence for the presence of hypoglycemic components in PFP. The major chemical components in PFP are flabelliferins and fibre (Thabrew and Jansz, 2004). In the previous study with mice conducted by Uluwaduge et al. (2005a), it was shown that flabelliferin-II can inhibit intestinal glucose uptake. However, the effect of fibre was not evaluated. It is possible that the fibre in PFP can also contribute significantly to reduce blood glucose level.

It is well documented that fibre can reduce intestinal glucose absorption by modulating the absorption of carbohydrate in the small intestine (Wickramanayake, 1998). For example, Guar gum, which is a gel forming dietary fibre (a commercially separated product of the endosperm of the Indian-cluster bean, (*Cyamopsis tetragonolobus*) has been shown to be a factor which reduces the fasting blood sugar levels significantly in type II diabetics (Usithupa et al., 1990).

PFP contains 11.9 g/100g of total crude fibre (Sumudini, 2002). In the present study with type-II diabetic patients, oral administration of a suspension of fibre isolated from PFP resulted in a small (2-11%)

statistically non significant ($p > 0.05$) decline in the blood glucose (after glucose load) concentration when compared with their own control values.

From the results of the present study and previous study with mice (Uluwaduge et al., 2005a), the major inhibitory effect of pinattu on intestinal glucose absorption appears to be mediated by the flabelliferin fraction (flabelliferin-II) rather than the fibre contained in PFP.

Since the study with mice conducted by Uluwaduge et al. (2005a) has shown that F-II can inhibit intestinal glucose uptake by inhibition of the intestinal Na^+/K^+ -ATPase pump, and increase the faecal excretion of glucose, the same mechanisms may be responsible for the PFP mediated reduction in blood glucose levels in human diabetic subjects also. Therefore, PFP (pinattu) may have a special application in the control of blood glucose in obese diabetic patients and in the weight control of obese individuals in general. To obtain evidence for the above hypothesis, a further study that includes a larger number of diabetic patients has to be conducted. The study must be followed up by a full scale multi-centre clinical trial that will enable a better statistical evaluation of results. It may be concluded from results of the present study that pinattu reduces the postprandial blood glucose levels in all the patients included in this study and therefore has the potential to be used as an anti-diabetic agent.

According to information in published literature there is a remarkable increase in the diabetic population of the world. Therefore, if F-II from PFP can be developed into a suitable pharmaceutical drug, it could be of benefit not only to diabetic patients of Sri Lanka, but also to the diabetic population of the world. The weight reducing properties of F-II may also be of benefit to patients suffering from cardio-vascular diseases.

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