Aqueous extracts of husks of *Plantago ovata* reduce hyperglycaemia in type 1 and type 2 diabetes by inhibition of intestinal glucose absorption

J. M. A. Hannan¹, L. Ali², J. Khaleque², M. Akhter², P. R. Flatt¹ and Y. H. A. Abdel-Wahab¹*

¹School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland, UK
²Department of Pharmacology, Biomedical Research Group, BIRDEM, Dhaka-1000, Bangladesh

(Received 2 December 2005 – Revised 1 March 2006 – Accepted 21 March 2006)

*Plantago ovata* has been reported to reduce postprandial glucose concentrations in diabetic patients. In the present study, the efficacy and possible modes of action of hot-water extracts of husk of *P. ovata* were evaluated. The administration of *P. ovata* (0.5 g/kg body weight) significantly improved glucose tolerance in normal, type 1 and type 2 diabetic rat models. When the extract was administered orally with sucrose solution, it suppressed postprandial blood glucose and retarded small intestinal absorption without inducing the influx of sucrose into the large intestine. The extract significantly reduced glucose absorption in the gut during *in situ* perfusion of small intestine in non-diabetic rats. In 28 d chronic feeding studies in type 2 diabetic rat models, the extract reduced serum atherogenic lipids and NEFA but had no effect on plasma insulin and total antioxidant status. No effect of the extract was evident on intestinal disaccharidase activity. Furthermore, the extract did not stimulate insulin secretion in perfused rat pancreas, isolated rat islets or clonal β cells. Neither did the extract affect glucose transport in 3T3 adipocytes. In conclusion, aqueous extracts of *P. ovata* reduce hyperglycaemia in diabetes via inhibition of intestinal glucose absorption and enhancement of motility. These attributes indicate that *P. ovata* may be a useful source of active components to provide new opportunities for diabetes therapy.

*Plantago ovata*: Glucose absorption: Gastrointestinal motility: Insulin secretion: Insulin action

Diabetes mellitus is a major cause of disability and hospitalization resulting in significant financial and social burden across the world (Foster, 1994). Although a sub-group of type 2 patients can be managed by diet alone, most individuals with diabetes require oral hypoglycaemic drugs or insulin. However, the effectiveness of drug therapy is limited and is often associated with complications and side-effects including hypoglycaemia (Davis & Granner, 1996). Many patients fail to respond to oral antidiabetic agents and consequently need insulin therapy. In recent years, the popularity of complementary medicine has increased. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine, are used commonly in India (Warier, 1995). WHO has also recommended evaluation of the effectiveness of these plants in situations where there is a lack of safe modern drugs (Upadhyay & Pandey, 1984). The antihyperglycaemic activity of a large number of these plants has been evaluated and confirmed in different animal models (Swanston-Flatt et al. 1991).

*Plantago ovata* (Psyllium) is found in India, Iran, northern Africa and Pakistan. It has been used traditionally for constipation, diarrhoea, haemorrhoids, irritable bowel syndrome, weight loss, obesity, high cholesterol and diabetes (Mehta et al. 1976). The seeds and the husks contain high levels of fibre and become highly gelatinous when soaked in water. This stimulates bowel evacuation and thus has been used widely as a fibre supplement in the treatment of constipation (Chopra et al. 1986). According to a report by the American Diabetic Association, fibre improves the control of blood glucose and delays glucose absorption and hyperinsulinaemia (Chandalia et al. 2000). Studies on the effect of *P. ovata* husk in patients with type 2 diabetes found that it can reduce the postprandial rise of glucose and insulin levels significantly (Wolfever et al. 1991; Anderson et al. 1999). The plant has been shown also to reduce carbohydrate absorption (Abraham & Mehta, 1988). It delayed gastric emptying and reduced colon transit time in man (Washington et al. 1998).

It is well known that type 2 diabetes is associated with a significantly increased risk of macrovascular disease (Laakso & Lehto, 1997). Supplementation of the diet with soluble fibre or consumption of a high-fibre diet has been shown to lower total serum cholesterol and triacylglycerol in type 2 diabetic patients (Vinik & Jenkins, 1988). *P. ovata* also reduced total cholesterol and LDL-cholesterol in animals (Fernandez et al. 1995; Terpstra et al. 2000) and in man (Romero et al. 1998; Burton & Manninen, 1982).

The present study was undertaken to evaluate the antidiabetic properties of the husk of *P. ovata* and to explore the possible mechanisms of action. The effects of the plant on glucose homeostasis, carbohydrate digestion, absorption and gastrointestinal motility were investigated. Possible effects on insulin secretion were also evaluated using different *in vitro* systems and glucose uptake was studied using 3T3 adipocytes.

* Corresponding author: Dr Yasser Abdel-Wahab, fax +44 (0)28 7032 4956, email y.abdel-wahab@ulster.ac.uk
Materials and methods

Plant materials and preparation of extract

Husks of *Plantago ovata* were collected from a local market in Bangladesh (north-eastern region) and botanically authenticated with voucher specimens deposited in the National Herbarium, Bangladesh. For the preparation of hot-water extract, *P. ovata* husk (200 g) was boiled with distilled water (44 litres) for 1 h and filtered. The filtrate was centrifuged at 4000 rpm (1750 g) and the supernatant was collected and concentrated by rotary evaporator. The extract was finally freeze-dried (21 g).

Animals and induction of type 1 and 2 diabetes

Long-Evans male rats were bred at BIRDEM (Bangladesh) and maintained on 12 h light–dark cycle at 21 ± 2°C. A standard pellet diet and water were supplied *ad libitum*. The overall nutrient composition of the diet was 36·2% carbohydrate, 20·9% protein, 4·4% fat and 38·5% fibre with metabolizable energy content of 11.8 MJ/kg (2820 kcal/kg). Type 1 diabetes was induced by a single intraperitoneal injection of anaesthetized, fasted rats (180–220 g) with 65 mg streptozotocin/kg body weight dissolved, immediately before use, in 0·5 M-citrate buffer (pH 4·5). The blood glucose level was checked on the seventh day after injection of streptozotocin. Animals having blood glucose levels >20 mmol/l were considered to be diabetic. Type 2 diabetes was induced by a single intraperitoneal injection of rats 48 h old with 90 mg streptozotocin/kg body weight (Bonner-Weir et al. 1981). Experiments were carried out 3 months after injection. During the experimental periods, the diabetic rat models did not receive any therapy to control diabetes other than aqueous extracts of *P. ovata*. In addition, the mean fasting plasma glucose concentrations recorded in type 1 and type 2 diabetic rat models prior to acute studies were 9.0 (SEM 0·5) and 26·6 (SEM 1·0) mmol/l, respectively (n 6; P<0·001).

Effects of aqueous extracts of *Plantago ovata* on glucose homeostasis

To evaluate effects on fasting blood glucose, the hot-water extract of *P. ovata* (0·5 g/kg body weight) was suspended in water and administered by gavage to 12 h fasted rats. The control group received an equal volume of deionized water and administered by gavage (0·5 g/kg body weight) twice daily for 28 d. Control rats were similarly administered water alone (10 ml/kg body weight). Blood samples were collected from the cut tip of the tail at the times indicated in the figures. Serum was separated by centrifugation and stored at −20°C until analysed.

Effects of *Plantago ovata* on sucrose absorption from gastrointestinal tract

Type 2 diabetic and control rats were fasted for 12 h before receiving a 50% sucrose solution by gavage (2·5 g/kg body weight) with or without hot-water extract of *P. ovata* (0·5 g/kg body weight). Blood samples were obtained from the tail vein before and 30, 60, 120 and 240 min after sucrose administration for the determination of glucose. Some of the rats were killed at these timings. The gastrointestinal tract was excised and divided into six segments: the stomach; the upper 20 cm, middle and lower 20 cm of the small intestine; the caecum; the large intestine. Each segment was washed out with acidified ice-cold saline and centrifuged at 3000 rpm (1000 g) for 10 min. The resulting supernatant was boiled for 2 h to hydrolyse the sucrose followed by neutralization with NaOH. Blood glucose and the amount of glucose liberated from residual sucrose in the gastrointestinal tract were measured. The gastrointestinal sucrose content was calculated from the amount of liberated glucose (Goto et al. 1995).

Effects of *Plantago ovata* on intestinal glucose absorption

An intestinal perfusion technique (Swintosky & Pogonowskawala, 1982) was used to study the effect of *P. ovata* on intestinal absorption of glucose in 36 h fasted non-diabetic rats anaesthetized using sodium pentobarbital (50 g/kg). The hot-water extract of *P. ovata* (10 mg/ml, equivalent to 0·5 g/kg) suspended in Krebs Ringer buffer, supplemented with glucose (54 g/l), was passed through pyloris and the perfusate was collected from a catheter inserted at the end of ileum. The control group was perfused with Krebs Ringer buffer supplemented with only glucose. Perfusion was carried out at a rate of 0·5 ml/min for 30 min at 37°C. The results were expressed as percentage of absorbed glucose, calculated from the amount of glucose in solution before and after the perfusion.

Effects of *Plantago ovata* on intestinal disaccharide activity and gastrointestinal motility

The hot-water extract of *P. ovata* was administered by a gavage (0·5 g/kg body weight) to 20 h fasted rats. After 60 min, the rats were killed and the small intestine was isolated, cut longitudinally, rinsed with ice-cold saline and homogenized with 10 ml saline (0·9% NaCl). Aliquots of homogenate were then incubated with 40 mmol/l sucrose at 37°C for 1 h. Disaccharidase activity was calculated by glucose concentration converted from sucrose as mmol/mg glucose per protein per h. For determination of gastrointestinal motility, the hot-water extract of *P. ovata* was given by gavage (0·5 g/kg body weight) to 12 h fasted non-diabetic rats. After 60 min, BaSO₄ milk (10% w/v in 0·5% sodium carboxymethyl cellulose suspension) was similarly administered. After a further 15 min, the rats were killed and gastrointestinal tract was excised. The distance traversed by BaSO₄ milk was measured and expressed as a percentage of the total length of small intestine.

Effects of *Plantago ovata* on insulin secretion and action

Long-Evans rats (180–250 g) were used for studies on insulin secretion from perfused pancreas and isolated islets. Pancreatic perfusion studies were carried out at 37°C according to the method of Giroix et al. (1983). Pancreatic islets were isolated by collagenase digestion (Moskalewski, 1969) and hot-water
Extract of *Plantago ovata* was tested at 30 µg/ml. BRIN-BD11 cells were used to evaluate the effects on insulin secretion from a pure cellular population. The production and characteristics of these cells have been described in detail elsewhere (McClenaghan *et al.* 1996). The glucose concentrations employed for pancreatic perfusion and islet studies were 3 and 11 mmol/l. For insulin release studies using BRIN-BD11 cells, the acute tests were carried out at 5.6 mmol/l glucose. The effects of *Plantago ovata* on cellular glucose uptake also employed a cell line, namely 3T3-L1 cells obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Glucose uptake was measured by adaptation of the method of Frost & Lane (1985).

**Analysis**

Glucose was measured by the glucose oxidase phenol and 4-amino-antipyrine method, HDL-cholesterol, NEFA and total antioxidant status were determined by enzymatic colorimetric methods using commercial kits from SERA PAK (Berkeley, CA, USA). Serum fructosamine was determined by a colorimetric method using a kit from Boehringer Mannheim GmbH (Mannheim, Germany). Insulin was measured by ELISA using a rat insulin kit from Crystal Chem Inc. (Downers Grove, IL, USA). Hepatic glycogen was extracted (Vander Vries, 1954) and measured at 650 nm in a microwell plate ELISA reader (EL 340; Bio-Tek, Winooski, VT, USA). Platelet aggregation was measured using a Chrono Log Lumi aggregometer (Chronolog Corp., Havertown, PA, USA) linked to a potentiometric recorder. Results are presented as means and standard deviations. Groups of data were compared using unpaired Student’s *t* test and Mann–Whitney *U*-test where appropriate. Where data were collected over a number of time-points, they have been analysed using repeated measures ANOVA, with Bonferroni adjustment to ensure an overall error rate of 5%. One-way ANOVA was performed and pair-wise comparisons to the control group were made using Dunnett’s test to preserve an overall error rate of 5%. Differences were considered significant at *P* < 0.05.

**Results**

**Acute and chronic effects of Plantago ovata on serum glucose and metabolic parameters**

Administration of *P. ovata* in the fasting state did not exert any hypoglycaemic action in either non-diabetic or diabetic (type 1 and type 2) rats (Fig. 1(A–C)). When given simultaneously with an oral glucose load, *P. ovata* significantly opposed the rise of serum glucose at 30 min (*P* < 0.01) in controls, type 1 and type 2 diabetic rats (Fig. 1(D–F)). Oral administration of sucrose caused a prominent elevation of serum glucose with a peak at 30 min in both normal and type 2 diabetic rats (Fig. 2). The rise in blood glucose after sucrose loading was suppressed significantly by *P. ovata* at 30 and 60 min in both groups of rats (*P* < 0.05). As shown in Table 1, oral administration of *P. ovata* to type 2 diabetic rats for 28 d lowered fructosamine levels with significantly (*P* < 0.05) compared with controls. No significant differences were observed in fasting levels of serum glucose, insulin and total antioxidant status. However, *P. ovata* significantly lowered total cholesterol (*P* < 0.05), triacylglycerol (*P* < 0.05) and NEFA (*P* < 0.01) levels. There were no significant differences in HDL, platelet aggregation, pancreatic insulin or hepatic glycogen content compared with the control (Table 1).

**Effects of Plantago ovata on sucrose absorption from the gastrointestinal tract**

Administration of hot-water extract of *P. ovata* (0.5 g/kg) with the sucrose load in non-diabetic rats increased the residual intestinal sucrose content significantly (*P* < 0.01) in the middle part of the intestine at 30 min (control v. *P. ovata*, 8.5 (SD 3.0) mg v. 18.8 (SD 6.2) mg) and in the entire small intestine at 60 min (upper 20 cm, 4.8 (SD 2.6) mg v. 15.1 (SD 6.8) mg; middle, 0.61 (SD 6.15) mg v. 15 (SD 7.0) mg; lower 20 cm, 1.1 (SD 0.6) mg v. 1.9 (SD 1.3) mg) and 120 min (1.6 (SD 0.4) mg v. 5.5 (SD 2.8) mg, 4.7 (SD 1.9) mg v. 12.1 (SD 4.2) mg and 0.7 (SD 0.32) mg v. 1.6 (SD 0.64) mg). The total sucrose content remaining in the gastrointestinal tract was increased significantly in *P. ovata*-treated rats compared with normal controls (*P* < 0.05; Fig. 3(A)). Similar effects of *P. ovata* were observed in type 2 diabetic rats after a sucrose load (*P* < 0.05, at 60 and 120 min; Fig. 3(B)).

**Effects of Plantago ovata on intestinal glucose absorption**

As shown in Fig. 4, intestinal glucose absorption in non-diabetic rats was almost constant during 30 min of perfusion. Addition of *P. ovata* to the glucose perfusate resulted in a substantial decrease in intestinal glucose absorption during the whole experimental period (*P* < 0.01).

**Effects of Plantago ovata on intestinal disaccharidase activity and gastrointestinal motility**

There were no significant differences on disaccharidase (sucrase) activity between non-diabetic controls and rats supplemented with *P. ovata* (data not shown). In contrast, the plant extract significantly increased gastrointestinal motility by 20%, assessed using BaSO₄ milk, compared with non-diabetic controls (*P* < 0.001).

**Effects of Plantago ovata on insulin secretion and action**

Hot-water extract of *P. ovata* did not affect insulin secretion from the perfused rat pancreas, isolated rat islets or clonal pancreatic β cell line, BRIN-BD11, at a range of concentrations tested (8, 40, 200, 1000 and 5000 µg/ml) (data not shown). Similarly, *P. ovata* did not affect glucose transport in 3T3 adipocytes in the absence or presence of 10⁻⁹ M-insulin (data not shown).

**Discussion**

*Plantago ovata* is commonly used in India, Pakistan and Bangladesh for the treatment of constipation, irritable bowel syndrome and diabetes. However, a very limited number of studies have evaluated antihyperglycaemic effects of the husk (Anderson *et al.* 1999). In the present study, the effects of hot-water extract of *Plantago ovata* on glucose homeostasis...
were evaluated using a number of model in vivo and cellular systems.

Postprandial hyperglycaemia is undesirable as it increases glycosylation products, such as methylglyoxal, which play a role in the development of diabetic vascular disease (Thornalley, 1996). Acute elevation of glucose also increases coagulation (Ceriello et al. 1996) and results in multiple disturbances in endothelial cell function (Haller, 1997; King et al. 1997). The present serum fructosamine data in type 2 diabetic rat models receiving Plantago ovata extract daily clearly demonstrate that the plant exhibits antihyperglycaemic properties in the previous 2–3 weeks. Unlike fasting glucose, which did not change serum fructosamine, it represents a stable measure that reflects the recent glycaemic environment.

When administered with an oral glucose load, P. ovata markedly improved glucose tolerance in normal rats and type 2 diabetic (C, F) rats. Fasting rats were given glucose (2.5 g/kg body weight) without (●) or with (○) hot-water extract of P. ovata (0.05 g/kg body weight). For details of procedures, see p. 132. Values are means with standard deviations depicted by vertical bars (n 6). Mean values were significantly different from those of the respective control group (repeated measures ANOVA, adjusted using a Bonferroni correction): *P<0.05; **P<0.01.

Since the glucose-lowering effect of P. ovata was clearly evident when simultaneously administered with glucose, inhibition of glucose absorption in the gut is a likely contributor to the mechanism of action (Lempcke, 1987; Vinik & Wing, 1990). It is well known that high-fibre diets improve glucose tolerance in diabetes (Fukagawa et al. 1990; Nutall, 1993). This effect may be due to retarded gastric emptying, increased intestinal transit, or modification of the secretion and action of digestive enzymes (Hannah & Howard, 1994). In the present study, we assessed various effects of Plantago ovata extract on carbohydrate digestion and absorption in the gut. When the extract was given by gavage simultaneously with sucrose solution to non-diabetic and type 2 diabetic rats, it suppressed the resulting rise of serum glucose and increased the unabsorbed sucrose content throughout the small intestine. These effects do not appear to be associated with inhibition of intestinal
disaccharidase activity, although loss of competitive effects on enzyme activity cannot be entirely ruled out. This suggests that reduction of sucrose absorption was not related to the enzyme activity as observed with α-glucosidase inhibitors (Hanefeld et al. 1991). However, the extract significantly reduced glucose absorption during in situ perfusion of small intestine. Thus, limitation of the glycaemic excursion by *P. ovata* is at least partly due to the retardation of carbohydrate absorption, which is consistent with the suggestion of Abraham & Mehta (1988). Interestingly, several early in vitro studies suggest that high concentrations of metformin also inhibit glucose absorption (Caspar & Creutzfeld, 1971; Lorch, 1971). The effects of *P. ovata* extract on gastrointestinal motility were also evaluated in non-diabetic rats. A significant increase in motility was noted, which supports the findings of others (Chopra et al. 1986; Marteau et al. 1994). Thus, a component of the antihyperglycaemic activity of *P. ovata* is probably due to increased motility of the gastrointestinal tract.

Dyslipidaemia, particularly low levels of HDL-cholesterol and high levels of total cholesterol, triacylglycerols and NEFA, is an important risk factor for atherosclerotic complications of diabetes (Laakso & Lehto, 1997). Elevated NEFA levels also lead to pancreatic β cell lipid overload, dysregulation of insulin secretion (Prenkli et al. 1992; Zhou & Grill, 1995) and apoptotic cell death (Maezler et al. 2001; Lupi et al. 2002). Raised NEFA contributes to many of the other major metabolic abnormalities in diabetes including decreases in glucose transport, glycogen synthesis and glucose oxidation (DeFronzo et al. 1992). Thus, a key goal in the management of diabetes is correction of dyslipidaemia (Vague, 2003). In the present study, administration of extract of *P. ovata* for 28 d lowered total cholesterol, triacylglycerol and NEFA levels in type 2 diabetic rats. The present findings are consistent with other investigations (Everson et al. 1992; Fernandez et al. 1995). In the present chronic study, there was no significant effect on glycogen deposition in the liver, which may reflect lack of effect of *P. ovata* on insulin secretion.

In conclusion, the present study has demonstrated that the hot-water extract of the husk of *P. ovata* exerts strong antihyperglycaemic actions in type 1 and type 2 diabetes by

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**Table 1.** Effects of 28 d treatment with *Plantago ovata* on glucose homeostasis and other metabolic features in type 2 diabetic rats (n 12)†

(Values are means and standard deviations)

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<th>Parameters</th>
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<th>Day 28</th>
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<tr>
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<td>Control</td>
<td><em>P. ovata</em></td>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Glucose (mmol/l per l)</td>
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<td>Fructosamine (μmol/l)</td>
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<td>Pancreatic insulin (nmol/g tissue)</td>
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<td>Liver glycogen (g/100 g)</td>
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<td>Platelet aggregation (%)</td>
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</table>

TAS, total antioxidant status.

Mean values were significantly different from those of the control group (unpaired t test): *P < 0.05.

† Diabetes was induced by injection of neonatal rats with 90 mg streptozotocin/kg body weight 3 months prior to the experiment. Hot-water extract of *P. ovata* was administered by gavage (0·5 g/kg body weight) twice daily for 28 d. For details of procedures, see p. 132.
retarding the absorption of ingested carbohydrate. It also served to increase gastrointestinal motility and reduce atherogenic lipids. Thus *P. ovata* may be a useful dietary adjunct for the treatment of hyperglycaemia as well as dyslipidaemia in type 2 diabetes.

**References**


Antidiabetic actions of Plantago ovata


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