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A Novel *Gymnema sylvestre* Extract Stimulates Insulin Secretion from Human Islets *In Vivo* and *In Vitro*

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Many plant-based products have been suggested as potential antidiabetic agents, but few have been shown to be effective in treating the symptoms of Type 2 diabetes mellitus (T2DM) in human studies, and little is known of their mechanisms of action. Extracts of *Gymnema sylvestre* (GS) have been used for the treatment of T2DM in India for centuries. The effects of a novel high molecular weight GS extract, Om Santal Adivasi, (OSA®) on plasma insulin, C-peptide and glucose in a small cohort of patients with T2DM are reported here. Oral administration of OSA® (1 g/day, 60 days) induced significant increases in circulating insulin and C-peptide, which were associated with significant reductions in fasting and post-prandial blood glucose. *In vitro* measurements using isolated human islets of Langerhans demonstrated direct stimulatory effects of OSA® on insulin secretion from human β-cells, consistent with an *in vivo* mode of action through enhancing insulin secretion. These *in vivo* and *in vitro* observations suggest that OSA® may provide a potential alternative therapy for the hyperglycemia associated with T2DM. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: Gymnema sylvestre extract; Type 2 diabetes mellitus; human islets of Langerhans; insulin secretion.

INTRODUCTION

The prevalence of diabetes mellitus is growing worldwide and it has been estimated that by the year 2020, 250 million people will be suffering from this disease, with the vast majority having Type 2 diabetes mellitus (T2DM) (O'Rahilly, 1997). The most common pharmacological treatments for T2DM, sulphonylureas and biguanides (Defronzo *et al.*, 2004), tend to lose their effectiveness with prolonged treatment duration, so new pharmacological agents have been developed, including peroxisome proliferator-activated receptor-γ (PPARγ) agonists and agents that stimulate insulin secretion such as glucagon-like peptide-1 (GLP-1) analogs and dipeptidyl peptidase IV (DPPIV) inhibitors (reviewed by Persaud and Jones, 2008). However, the widespread application of these novel agents is limited by their cost and by concerns about their long-term safety (VanDeKoppel *et al.*, 2008). Herbal medicines have attracted attention as alternative therapeutic agents for treating T2DM because they are relatively inexpensive and many have been used for decades or centuries without deleterious side effects. However, while many plant-based products have been suggested as potential antidiabetic agents, few have yet been shown to be effective in treating the symptoms of T2DM in humans, and their mechanisms of action are uncertain.

Gymnema sylvestre (GS) is a large woody climber plant, from the Asclepiadaceae family, and is native to central and western India, tropical Africa and Australia. This plant has been used in Ayurvedic medicine for the treatment of diabetes in India for centuries. Crude or low molecular weight GS extracts have been reported to have antidiabetic effects in alloxan- or streptozotocin-treated animals by raising plasma insulin levels and attenuating blood glucose responses during oral glucose/sucrose tolerance tests (Gupta, 1961, 1963; Gupta and Variyar, 1961; Mhaskar and Caius, 1930; Okabayashi *et al.*, 1990; Shanmugasundaram *et al.*, 1983; Srivastava *et al.*, 1985, 1986; Terasawa *et al.*, 1994). Similarly, crude or low molecular weight GS extracts have been reported to have hypoglycemic effects in patients with hyperglycemic diabetes (Baskaran *et al.*, 1990; Khare *et al.*, 1983; Shanmugasundaram *et al.*, 1981, 1990). The antihyperglycemic effect of GS extracts in these studies was postulated to be due, at least in part, to the ability of the plant leaves to increase insulin secretion from β-cells of the islets of Langerhans, although this was not directly demonstrated.

Most biologically active compounds isolated from GS leaves have relatively low molecular weights (Murakami *et al.*, 1996; Sinsheimer *et al.*, 1970; Sinsheimer and Rao, 1970; Sugihara *et al.*, 2000), and the previously documented glucose-lowering activities of GS have been attributed to these low molecular weight components.

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Recently a high molecular weight GS extract was isolated (Chatterji, 2005a, 2005b) which was subsequently designated as the OmSantal Adivasi extract (OSA®) after the Santal tribe who first used GS leaves in Ayurvedic medicine. In a recent study it was demonstrated that OSA® stimulated insulin secretion from the mouse MIN6 β-cell line in *vitro* (Liu *et al.*, 2009) without exerting the deleterious effects on β-cell viability and membrane integrity that are associated with crude GS extracts (Persaud *et al.*, 1999). The present study investigated the effects of oral OSA® on plasma insulin, C-peptide and glucose in a small cohort of patients with T2DM, and determined whether OSA® acts by direct effects on human pancreatic β-cells.

MATERIALS AND METHODS

Materials. All reagents and chemicals were purchased from Sigma Chemical Co (Dorset, UK) unless otherwise stated. $Na^{125}I$ for insulin iodination was from Perkin Elmer (Bucks, UK). Human islets were provided for this study, with appropriate ethical approval, by the Human Islet Isolation Unit at King's College Hospital, London, UK. Islets were isolated from whole human pancreas by collagenase digestion as described previously (Huang *et al.*, 2004). Briefly, pancreata were removed from non-diabetic heart beating organ donors with permission from donor relatives and approval from the Ethical Committee of King's College Hospital. Islets were maintained in culture in Connaught Medical Research Laboratories (CMRL) medium supplemented with 10% FCS, 100 U/mL penicillin/0.1 mg/mL streptomycin and 2 mM glutamine at 37°C (95% O_2/5% CO_2).

Plant material and preparation. The GS extract used in this study (OSA®) was prepared by extracting fresh GS leaves by aqueous alcohol according to protocols described in the US Patents 6949261 and 6946151 (Chatterji, 2005a, 2005b). GS leaves were identified by a botanist, and a voucher specimen (reference GS1-OSA1-G123/C) was deposited with Ayurvedic-Life International LLC (Neenah, WI 54946-0010, USA). The OSA® extract used in this study was a gift from Ayurvedic Life International LLC, Wisconsin, USA. OSA® solutions were freshly prepared for *in vitro* experiments as a 200 mg/mL stock in water, and then diluted as appropriate using a physiological salt solution (Gey and Gey, 1936).

Patient cohort. Eleven patients (7 female, 4 male) were recruited and consented for an *in vivo* study of the effects of OSA® administration on blood glucose and insulin levels. The mean age of the cohort was 50.1 ± 3 years (female: 50.7 ± 4; male: 49 ± 5, $p > 0.2$) with a range of 36–70 years. The mean body weight at the outset of the trial was 58.7 ± 7.6 kg (female: 57.7 ± 3.0; male: 60.5 ± 2.8, $p > 0.2$) with a range of 45–70 kg. Patients were recruited, following local ethical approval, from Burdwan Medical College clinic, West Bengal, and were either newly diagnosed with T2DM, or had previously been treated with standard pharmacological regimens. Exclusion criteria were pregnancy, pre-existing heart disease, hypertension or respiratory disorders, and failure of compliance with the protocol.

Treatment and analysis. OSA® was administered orally in capsule form at a dose of 500 mg (2 × 250 mg capsules) two times each day before food intake for 60 days, giving a total daily dose of 1 g OSA®. Blood samples were taken at the start of the trial (day 0) and at the completion of the trial (day 60), and body weight was recorded at the start and completion of the trial. Blood glucose was estimated as described (Middleton and Griffiths, 1957) and insulin and C-peptide were measured in serum samples by radioimmunoassay (RIA), as described (Claudio and Lagua, 1995).

Insulin secretion from human islets of Langerhans. The effect of OSA® on insulin secretion from human islets *in vitro* was examined using a multi-channel, temperature-controlled perifusion system, essentially as described previously (Liu *et al.*, 2009). The perifusion system consisted of 16 Swinnex chambers fitted with 1 μm nylon mesh filters. Islets within the chambers were perifused at a flow rate of 0.5 mL/min with a physiological buffer (Gey and Gey, 1936) supplemented with 2 mM $CaCl_2$, 2 mM glucose and 0.5 mg/mL BSA for 60 min to establish a stable basal rate of insulin secretion, after which perifusate samples were collected at 2 min intervals and stored at –20°C until assayed for insulin content by radioimmunoassay (Jones *et al.*, 1988). Since glucose-induced insulin secretion is temperature-dependent, all perifusion experiments were carried out in a temperature-controlled room at 37°C.

Statistical analysis. Data are represented as mean ± SEM unless otherwise stated. Differences between treatment groups were assessed using analysis of variance (ANOVA) and Bonferroni's *t*-test for multiple comparisons or Student's paired *t*-test (two tailed), as appropriate. Differences between treatment groups were considered significant when $p < 0.05$.

RESULTS

Effects of OSA® administration *in vivo*

Daily oral administration of OSA® for 60 days produced significant improvements in glycemic control, as shown in Fig. 1. Thus, in 10/11 patients OSA® treatment was associated with a reduction in fasting blood glucose levels (Fig. 1A), with a mean reduction from 162 ± 23 to 119 ± 17 mg/dL ($p < 0.005$). Post-prandial blood glucose levels (Fig. 1B) also showed significant reductions in 10/11 patients, with a reduction from 291 ± 10 to 236 ± 30 mg/dL ($p < 0.02$). OSA® treatment had no significant effect on the extent of the post-prandial excursion in plasma glucose (day 0, 132 ± 10 mg/dL; day 60, 117 ± 26 mg/dL, $p > 0.2$), nor on body weight over the 60 day period of the trial (day 0, 58.7 ± 7.6 kg; day 60, 53.9 ± 7.3 kg, $p > 0.2$). One patient showed no reduction in either fasting or post-prandial blood glucose levels and no change in body weight during the treatment period.

The improvements in glycemic control in response to OSA® treatment were associated with increased circulating levels of insulin and/or C-peptide in all patients, as shown in Fig. 2. Thus 60 days treatment with OSA® induced a mean increase in serum insulin (Fig. 2A) from 24 ± 9 to 32 ± 6 μU/mL ($p < 0.001$), with a corresponding

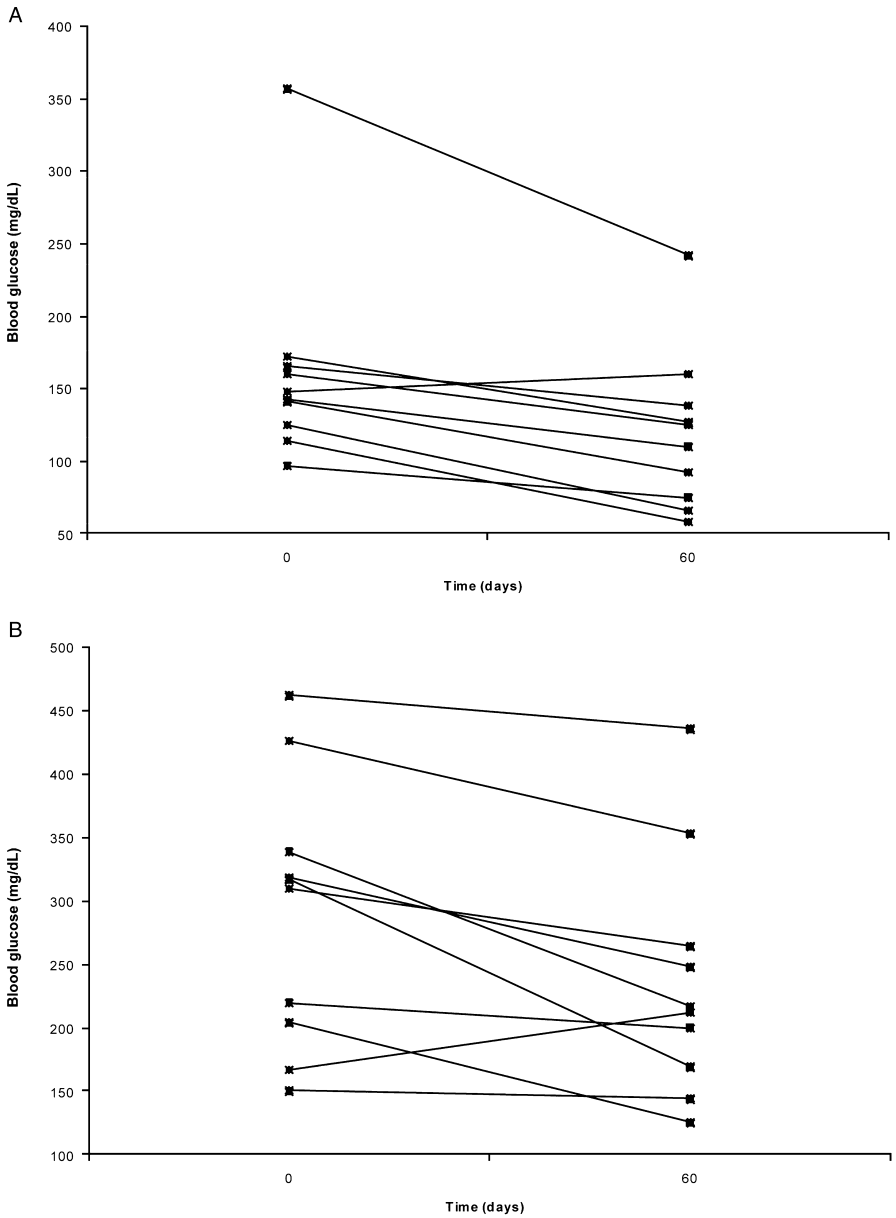

Figure 1. Effect of OSA® on fasting and post-prandial blood glucose levels of patients with Type 2 diabetes mellitus. OSA® (1000 mg/day) was administered orally and fasting (A) and post-prandial (B) blood glucose levels were determined at the start ($t = 0$ day) and completion ($t = 60$ days) of the study as described in the Materials and Methods. Data show blood glucose levels of individual patients in mg/dL at two time points (0, 60 days). Both fasting and post-prandial glucose levels were significantly reduced ($p < 0.02$) after 60 days of treatment with OSA®.

increase in serum C-peptide (Fig. 2B) from 298 ± 42 to 447 ± 48 pmol/L ($p < 0.05$).

Effect of OSA® on insulin secretion from isolated human islets

The effects of OSA® on the pattern and rate of insulin secretion, and the reversibility of its effects, were tested using human islets in *vitro*, as shown in Fig. 3. Perifusion of human islets with buffer supplemented with 0.125 mg/mL OSA® at a sub-stimulatory concentration (2 mM) of glucose evoked an approximately 2-fold increase in insulin secretion (217 ± 18% basal, $p < 0.001$, $n = 4$), as shown in Fig. 3A. The response to OSA® was rapid in onset, sustained for the duration of exposure to OSA®, and rapidly reversible upon its withdrawal. Subsequent exposure to 20 mM glucose following OSA® treatment induced a further increase in insulin secretion (Fig. 3A).

In addition to initiating an insulin secretory response, OSA® (0.125 mg/mL) potentiated glucose-induced insulin secretion, as shown in Fig. 3B. Thus, increasing the glucose concentration from 2 to 20 mM (10–30 min) resulted in the expected biphasic pattern of glucose-induced insulin secretion. The first phase was rapid and transient, reaching a peak within 4 min followed by a sustained second phase of insulin secretion of a lower magnitude. Exposure to OSA® (0.125 mg/mL) in the continued presence of 20 mM glucose (30–50 min) further potentiated the glucose-induced secretory response, with enhanced rates of insulin secretion being maintained for the duration of exposure to OSA®.

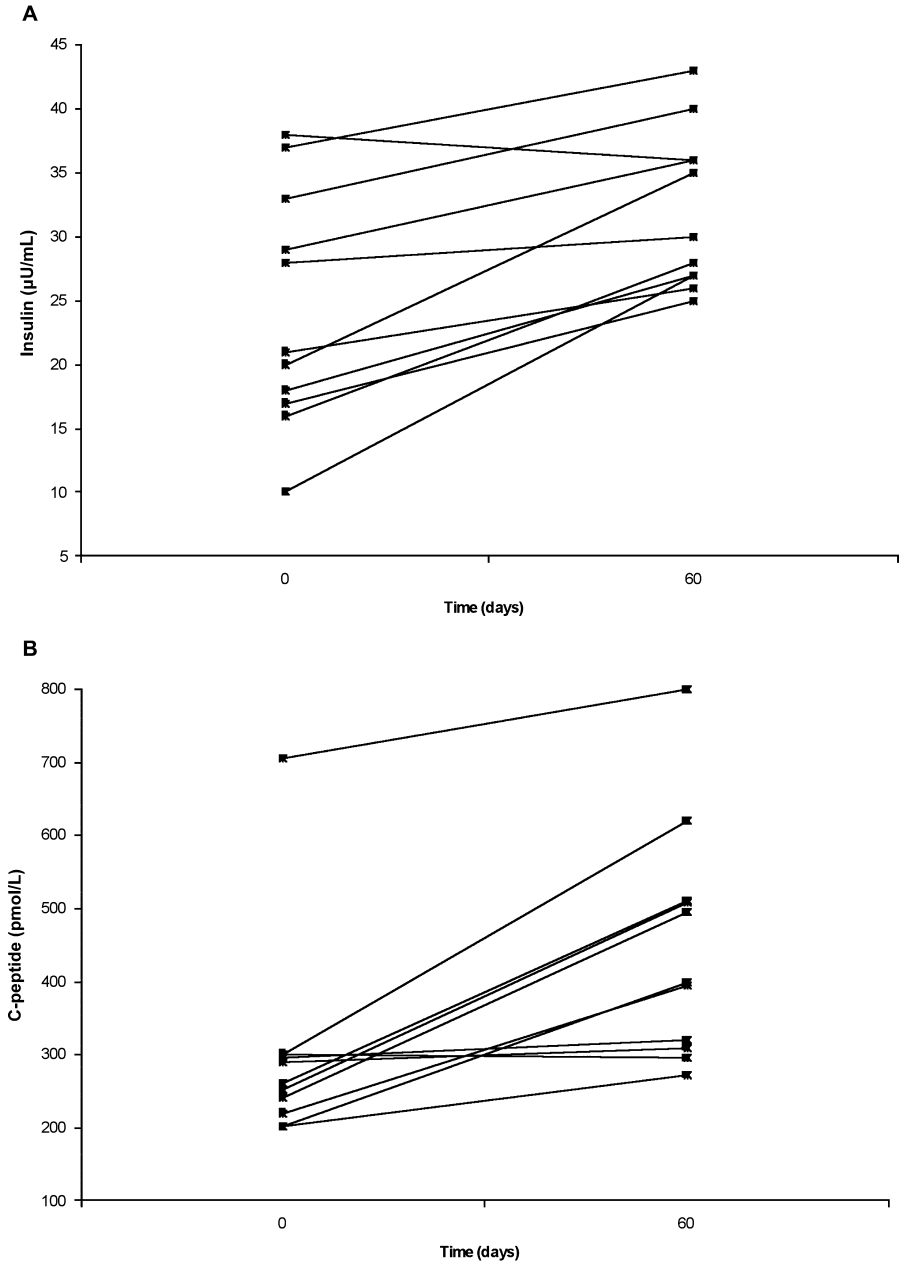

Figure 2. Effect of daily oral administration of OSA® on plasma insulin and C-peptide levels of patients with Type 2 diabetes mellitus. Administration of OSA® (1000 mg/day) for 60 days significantly ($p < 0.05$) increased circulating levels of insulin (A) and C-peptide (B). Data show the plasma levels of insulin (μU/mL) and C-peptide (pmol/L) for each patient before and after treatment.

DISCUSSION

Crude extracts of GS leaves or low molecular weight isolates from GS extracts have been reported to reduce hyperglycemia without altering the insulin sensitivity of target tissues in animal models of diabetes (Gupta, 1961, 1963; Gupta and Variyar, 1961; Mhaskar and Caius, 1930; Okabayashi *et al.*, 1990; Shanmugasundaram *et al.*, 1983; Srivastava *et al.*, 1985, 1986; Sugihara *et al.*, 2000; Terasawa *et al.*, 1994; Tominaga *et al.*, 1995). We have previously reported the isolation of a high molecular weight GS extract of >3000 Da, as determined by molecular weight cut-off filtration (Chatterji, 2005a, 2005b), and we have recently demonstrated that this extract, designated OSA®, had a direct stimulatory effect on insulin-secreting β-cells (Liu *et al.*, 2009) without inducing the cell damage associated with crude GS extracts *in vitro* (Persaud *et al.*, 1999). To date, there have been relatively few reported studies of any GS extracts in humans (Baskaran *et al.*, 1990; Khare *et al.*, 1983; Shanmugasundaram *et al.*, 1981, 1990), and no reported studies in humans using OSA® or other high molecular weight isolates. The present study therefore investigated whether this novel isolate from GS leaves had an effect on clinically relevant parameters such as insulin, C-peptide and glucose levels in a small cohort of patients with T2DM. In addition, isolated human islets of Langerhans were used to investigate whether OSA® had direct stimulatory effects on insulin secretion from islet β-cells.

The patients recruited to this study presented with both fasting and post-prandial hyperglycemia but with circulating insulin and C-peptide levels within the normal range. Oral treatment with OSA® for 60 days

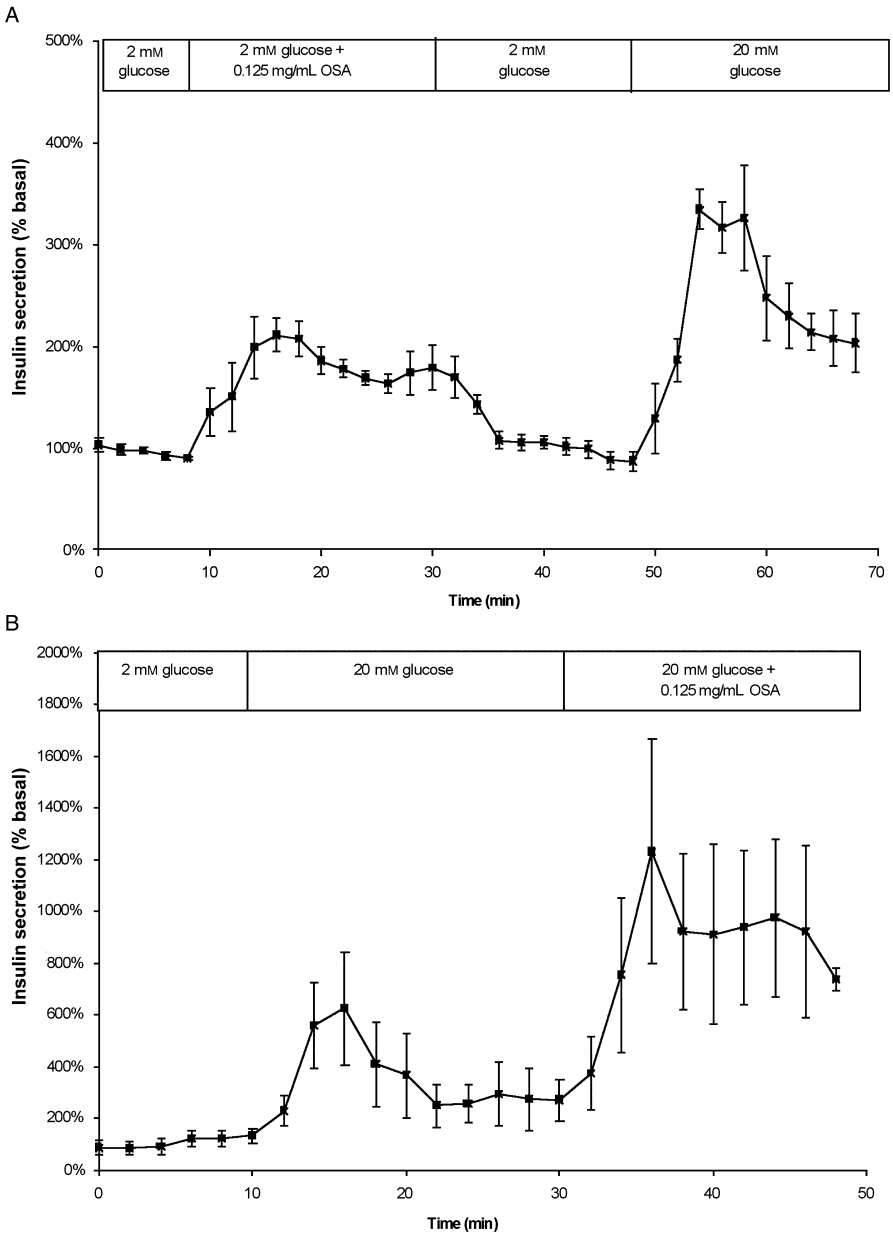

Figure 3. Effect of OSA® on insulin secretion from isolated human islets. Human islets were perifused with buffer supplemented with 2 mM or 20 mM glucose in the absence or presence of 0.125 mg/mL OSA®, as shown by the horizontal bar. Fractions were collected every 2 min and insulin content was determined by RIA. Data are expressed as % increase in insulin secretion over basal (2 mM glucose). Insulin secretion was significantly stimulated by the presence of 0.125 mg/mL OSA® at both 2 mM (A) ($p < 0.001$) and 20 mM (B) ($p < 0.05$) glucose. Points show mean ± SEM, $n = 4$.

resulted in significant reductions in fasting and post-prandial plasma glucose levels to nearly normal values in 10 of 11 patients, although it is unclear why one member of the cohort did not respond to OSA® treatment. The effect of OSA® to decrease plasma glucose was not associated with changes in post-prandial glucose excursions nor in body weight, indicating that the effect of OSA® on glycemia was not secondary to a decrease in either glucose absorption or food intake, consistent with an effect via enhanced insulin secretion. In accordance with this, measurements of plasma insulin and C-peptide levels demonstrated that OSA®-induced improvements in glycemic control were accompanied by elevations in plasma insulin and C-peptide concentrations suggesting that OSA® has a direct stimulatory effect on β-cells in the islets of Langerhans.

To further test this hypothesis, the effects of OSA® on the rate and pattern of insulin secretion from isolated human islets were examined *in vitro*. Our demonstration that OSA® stimulated insulin secretion from isolated human islets is consistent with a direct effect on human β-cells of a component(s) of the high molecular weight GS extract, and offer a mode of action through which oral treatment with OSA® resulted in increases in circulating insulin and C-peptide levels, and thus reduced fasting and post-prandial blood glucose. In our *in vitro* experiments OSA® initiated insulin secretion at a sub-stimulatory concentration (2 mM) of glucose suggesting a nutrient- and metabolism-independent mechanism of action on human β-cells. This was further confirmed by our observations that OSA® also potentiated the maximum glucose-induced (20 mM) secretory

responses, indicating that OSA® does not act as a nutrient itself, nor as an enhancer of glucose metabolism, but has an effect independent of nutrient metabolism.

Extracts of GS leaves contain a mixture of saponin compounds (Murakami *et al.*, 1996; Sinsheimer *et al.*, 1970; Sinsheimer and Rao, 1970; Sugihara *et al.*, 2000) and the presence of these compounds may be detrimental to membrane integrity and cell viability (Schulz, 1990), causing pathological and unregulated release of insulin from damaged β-cells (Persaud *et al.*, 1999). There are a number of reasons why this is unlikely to account for the insulin-releasing properties of OSA®. Firstly, in the *in vivo* study the saponins in the OSA extract most likely would be degraded in the gastrointestinal tract and therefore lose their detergent properties and membrane-damaging effects. Secondly, the OSA® fraction prepared from GS extract by molecular weight cut-off filtration has a low saponin content, and does not damage β-cell membranes at the concentrations used in the present study (Liu *et al.*, 2009). In accordance with this, our preliminary experiments demonstrated that at concentrations of ≤0.25 mg/mL, OSA® stimulated insulin secretion from islets and β-cells without compromising plasma membrane integrity as assessed by Trypan Blue exclusion test. Thirdly, insulin secretory responses induced by OSA® were sustained and readily reversible following its removal, consistent with the activation of a regulated secretory response. Finally, human islets which had been exposed to OSA® alone were capable of mounting a normal secretory response to glucose following OSA® treatment, confirming that OSA® treatment was not associated with β-cell damage because the cells were subsequently able to metabolize glucose and trigger membrane depolarization in response to glucose.

In summary, this is the first report showing that OSA®, a high molecular weight fraction isolated from GS leaf extract is effective in reducing blood glucose and increasing plasma insulin and C-peptide levels in humans. Our parallel *in vitro* studies suggest that at least some of these effects of OSA® can be attributed to a direct stimulatory effect on insulin secretion from β-cells in the islets of Langerhans. OSA® may therefore provide a potential alternative therapy for the hyperglycemia associated with T2DM.

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Duality of interest

The authors are not aware of any conflict of interest.

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