

# Insulin secretory actions of extracts of *Asparagus racemosus* root in perfused pancreas, isolated islets and clonal pancreatic $\beta$ -cells

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## Abstract

*Asparagus racemosus* root has previously been reported to reduce blood glucose in rats and rabbits. In the present study, the effects of the ethanol extract and five partition fractions of the root of *A. racemosus* were evaluated on insulin secretion together with exploration of their mechanisms of action. The ethanol extract and each of the hexane, chloroform and ethyl acetate partition fractions concentration-dependently stimulated insulin secretion in isolated perfused rat pancreas, isolated rat islet cells and clonal  $\beta$ -cells. The stimulatory effects of the ethanol extract, hexane, chloroform and ethyl acetate partition fractions were potentiated by glucose, 3-isobutyl-1-methyl

xanthine IBMX, tolbutamide and depolarizing concentration of KCl. Inhibition of *A. racemosus*-induced insulin release was observed with diazoxide and verapamil. Ethanol extract and five fractions increased intracellular  $\text{Ca}^{2+}$ , consistent with the observed abolition of insulin secretory effects under  $\text{Ca}^{2+}$ -free conditions. These findings reveal that constituents of *A. racemosus* root extracts have wide-ranging stimulatory effects on physiological insulinotropic pathways. Future work assessing the use of this plant as a source of active components may provide new opportunities for diabetes therapy.

*Journal of Endocrinology* (2007) **192**, 159–168

## Introduction

Diabetes mellitus (DM) is a major cause of disability and hospitalization that presents a significant burden on societies worldwide (Roglic *et al.* 2005, Booth *et al.* 2006). By the year 2010, the total number of people with DM is projected to reach more than 300 million. In Asia and Africa, there are limitations on presently available therapeutic options for diabetes, such as oral hypoglycaemic agents and insulin (Prout 1974, Holman & Turner 1991, Kameswara Rao *et al.* 1997). In such circumstances, herbal medicines for the treatment of diabetes become significant. Available literature reveals that more than 400 plant species have been claimed to have anti-hyperglycaemic activity (Mukherjee 1981, Oliver-Bever 1986, Atta-Ur-Rahman & Zaman 1989, Ivorra *et al.* 1989, Rai 1995). The recent explosion in the area of herbal medicine has led to a resurgence of nutritional, clinical and scientific interest in the potential of plant treatments for diabetes across the world (Swanston-Flatt *et al.* 1991a,b, Gray & Flatt 1997).

*Asparagus racemosus* (Liliaceae) is a popular vegetable consumed in many parts of the world and grows naturally throughout India, Asia, Australia and Africa. It is commonly used for the treatment of diarrhoea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac (Nadkarni 1976, Chadha 1985). The root of the plant has also been claimed by traditional healers to possess antidiabetic properties.

Studies on the extracts of *A. racemosus* have revealed a wide range of biological activities. These include antimutagenic, antitumor, antifungal (Edenharder 1990, Shimoyamada *et al.* 1990, Shao *et al.* 1996), diuretic (Balansard & Rayband 1987) and immunostimulatory effects (Thatte & Dahanukar 1988, Rege *et al.* 1999, Dhuley 1997). *A. racemosus* has been considered to be a lactagogue in lactational inadequacy (Sharma *et al.* 1996) and useful to decrease post-operative adhesions (scars; Rege *et al.* 1999). The protective effects of *A. racemosus* against the myelosuppression with single and multiple doses of cyclophosphamide have also been demonstrated (Thatte & Dahanukar 1988). Asparagus roots inhibited the growth of human leukaemia HL-60 cells (Shao *et al.* 1996) and more recently *A. racemosus* has been shown to exert antioxidant properties in rat liver mitochondrial membranes (Kamat *et al.* 2000).

The chemical constituents of *A. racemosus* have been studied to some extent. The compounds so far reported include flavonoids, oligosaccharides, amino acids, sulphur-containing acids and steroidal saponins (Shao *et al.* 1996). Various reports suggest that polysaccharides derived from the plant exhibit antioxidant as well as radioprotective properties (Gang *et al.* 1997, Liu *et al.* 1997a,b, Zeng *et al.* 1997). The polysaccharide kreskin also has been shown to have inhibitory effects on the oxidation of low density lipoprotein (LDL; Liu *et al.* 1997a,b). However, most studies evaluate mixtures of constituents and the fresh root juice of *A. racemosus* has been found to be effective in dyspepsia, being associated with anti-ulcerogenic activity (De *et al.* 1997, Sairam

*et al.* 2003). It has been reported that asparagus decreases gastric emptying time (Dalvi *et al.* 1990). Other studies have shown that the methanolic extracts of asparagus root reduced intestinal propulsive movement, castor oil-induced diarrhoea and intestinal fluid accumulation (Rege *et al.* 1999, Nwafor *et al.* 2000).

As well as claims by traditional healers that the roots of *A. racemosus* have antidiabetic properties, studies have reported reduced blood glucose level in rats and rabbits (Akhtar & Shah 1993, Rana *et al.* 1994). However, the mechanism of action has not yet been elucidated. In the present study, the effects of ethanol extract and five partition fractions of *A. racemosus* roots were evaluated on insulin secretion using the perfused rat pancreas, isolated rat islets and clonal  $\beta$ -cells.

## Materials and Methods

### *Plant materials and preparation of extract and fractions*

Dried roots of *A. racemosus* were purchased from the Ramkrishna Mission, Kolkata, India and botanically authenticated. Voucher specimens were deposited in the National Herbarium, Bangladesh. The roots were dried in an oven at 40 °C and ground into a fine powder. The powder (2 kg) was extracted with 80% ethanol (10 l) in a stainless steel extraction tank for approximately 4 days at room temperature by changing ethanol daily. The combined extract was filtered and evaporated to dryness using rotary evaporator. A membrane pump was used to evacuate the extract in order to remove the residual solvent. The extract was finally freeze-dried (330 g) using a Varian 801-model LY-3-TT freeze-dryer (USA). The dry sample was stored at 4 °C in a freezer. The ethanol extract (414 g) was subsequently partitioned between hexane (0.5 l × 3) and water (0.5 l). Hexane was separated and evaporated to dryness to get hexane-soluble material (1.64 g). The aqueous layer was further partitioned by chloroform (0.5 l × 3), ethyl acetate (0.5 l × 3) and 1-butanol (0.5 l × 3). These were finally evaporated to dryness to get chloroform (2.9 g), ethyl acetate (1.9 g) and 1-butanol (59 g) soluble materials. The residual aqueous part was condensed by rotary evaporator and finally freeze-dried (342 g). Figure 1 briefly outlines the preparation of ethanol extract and five partition fractions of dried roots of *A. racemosus*. Freeze-dried extract and fractions were subsequently reconstituted in Krebs–Ringer bicarbonate buffer prior to evaluation of the effects of *A. racemosus* on insulin release from perfused pancreas, isolated islets and clonal  $\beta$ -cell line (BRIN-BD11).

### *Effects of A. racemosus on insulin secretion from perfused pancreas*

Long–Evans rats (180–250 g) were anesthetized with sodium pentobarbital solution (50 mg/kg, i.p.) and the pancreas was isolated and perfused at 37 °C according to the method of Giroux *et al.* (1983). Extract and fractions of *A. racemosus* were dissolved in KRB buffer (pH 7.4): 118 mM NaCl, 4 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>,

25 mM NaHCO<sub>3</sub>, 1.25 g/l BSA and 40 g/l dextran T70) containing 2.8 mM or 11.1 mM D-glucose. The perfusate was continuously gassed with a mixture of O<sub>2</sub>:CO<sub>2</sub> (95:50). After a 20-min equilibration period, the composition of the perfusate was changed as indicated in Fig. 1. Effluent samples were frozen and stored at –20 °C for insulin assay.

### *Effects of A. racemosus on insulin secretion from isolated islets*

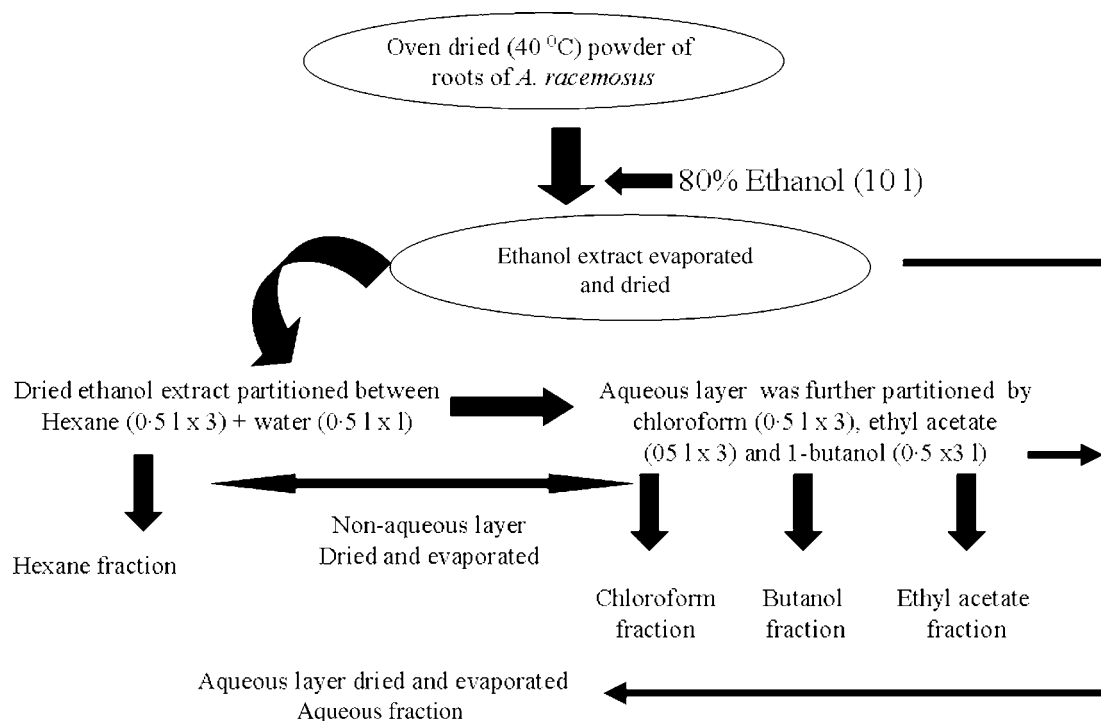
Islets were isolated from pancreas of Long–Evans rats (180–250 g body weight (bw)) by collagenase digestion (Moskalewski 1969). Following pre-incubation for 40 min in KRB containing 3 mM glucose, batches of 8–10 islets were incubated for 1 h at 37 °C in 400  $\mu$ l buffer containing either 3 or 11.1 mM glucose and either extracts or fractions of *A. racemosus* as shown in Table 1. Aliquots of supernatant were frozen and stored at –20 °C for insulin assay.

### *Effects of A. racemosus on insulin secretion and intracellular Ca<sup>2+</sup> from clonal $\beta$ -cells*

Clonal BRIN-BD11 cells were used to evaluate the mechanism of action of *A. racemosus* on insulin secretion and intracellular Ca<sup>2+</sup>. The origin and characteristics of this rodent cell line are described in detail elsewhere (McClenaghan *et al.* 1996). Cells were seeded in the multiwell plates at a concentration of 0.15 × 10<sup>6</sup> and allowed to attach overnight. Following pre-incubation for 40 min in KRB supplemented with 1.1 mM glucose, cells were incubated for 20 min at 37 °C with KRB supplemented with glucose, plant extracts and other reagents as indicated in the figures. Aliquots were removed from each well and stored at –20 °C for insulin assay. In another series of experiments, the effects of *A. racemosus* on intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) were determined using monolayers of BRIN-BD11 cells seeded in 96-well black-walled, clear bottom microplates (Greiner). Cells were washed with KRB and loaded with FLEXstation calcium assay reagents (Molecular Devices, Sunnyvale, CA, USA), for 10-min incubation at 37 °C (Miguel *et al.* 2004). Fluorometric data during subsequent exposure to extract and test agents indicated in Fig. 5 were acquired at a wavelength of 525 nm using the FLEXstation (Molecular Devices).

### *Statistical analysis*

For perfusion and islet studies, insulin was measured by ELISA using kits supplied by Crystal Chem. Inc (Downers Grove, IL, USA). In studies using BRIN-BD11 cells, insulin was measured by RIA (Flatt & Bailey 1981). The protein content of islets was determined using detergent compatible/protein kit supplied by Bio-Rad. Cell viability was evaluated by modified neutral red assay (Hunt *et al.* 1987). Results are presented as mean  $\pm$  S.D. for a given number of observations (*n*). Data from each set of observations were compared using unpaired Student's unpaired *t*-test and Mann–Whitney *U*-test where appropriate (SPSS for Windows). One-way ANOVA was performed and comparisons with the control group made



**Figure 1** Preparation of ethanol extract and five partition fractions (hexane, ethyl acetate, butanol, chloroform and aqueous fractions) of dried roots of *A. racemosus*.

using Dunnett's test to preserve an overall error rate of 5%. Differences were considered significant if  $P < 0.05$ .

## Results

### *Effects of A. racemosus on insulin secretion from perfused pancreas*

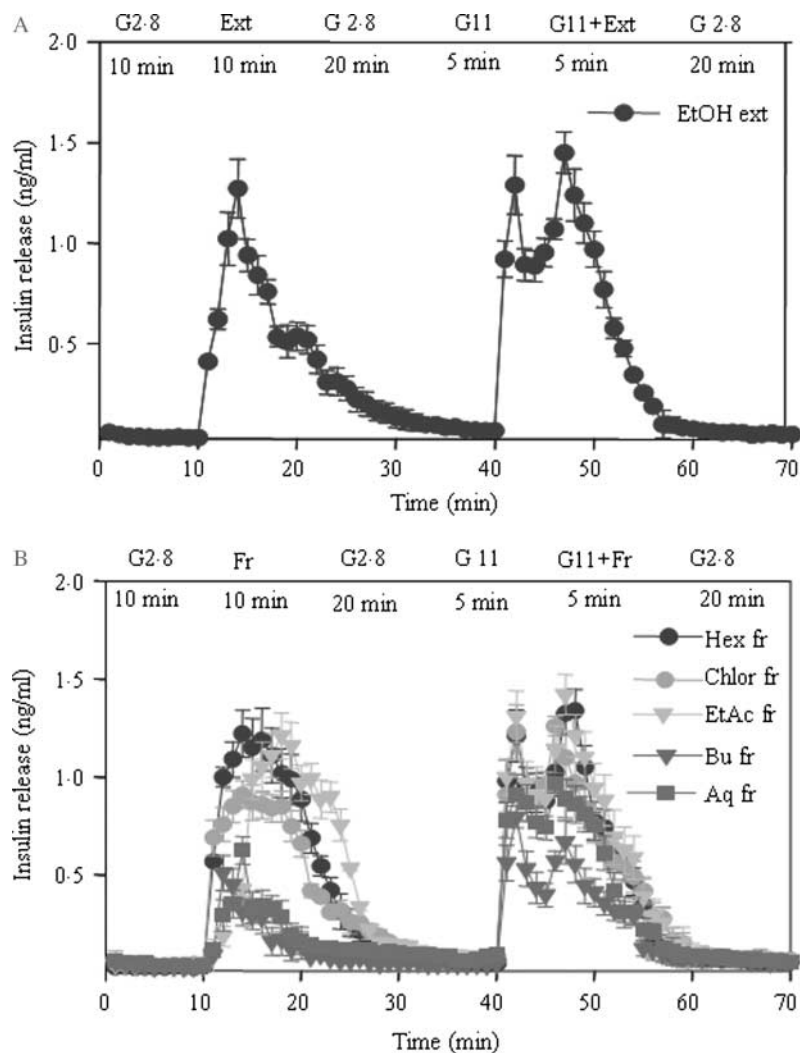
Ethanol extract caused a significant ( $P < 0.001$ ) increase in insulin release during 10-min perfusion, with a 21-fold increase

above basal ( $0.06 \pm 0.01$  ng/ml at 2.8 mM glucose (basal) vs  $1.27 \pm 0.09$  ng/ml with ethanol extract; Fig. 2A, Table 2). Subsequent exposure for 5 min to 11.1 mM glucose caused steep elevation in insulin release. When extract was reintroduced at 11.1 mM glucose, there was a further enhancement of insulin release ( $P < 0.05$ ). As shown in Fig. 2B and Table 2, perfusion with hexane, chloroform and ethyl acetate fractions evoked a significant increase in insulin release in an almost similar pattern, with a peak increase above basal of 36-, 18- and 28-fold respectively (2.8 mM glucose (basal)  $0.05 \pm 0.01$  ng/ml, hexane  $1.2 \pm 0.2$  ng/ml, chloroform  $0.9 \pm 0.1$  ng/ml, ethyl acetate

**Table 1** Effects of ethanol extract and five partition fractions of *A. racemosus* on insulin secretion from isolated rat islets. Data are presented as median (range),  $n = 8$

Group	Grams obtained from 1 kg starting materials (g/kg)	Insulin (ng/mg islet protein)	
		Glucose: 3 mM	Glucose: 11 mM
Control ( $n = 6$ )	No extract/fractions	2.99 (2.65–4.27)	5.41 (4.91–9.27) <sup>†</sup>
80% ethanol extract ( $n = 6$ )	165	5.01 (2.91–7.16)*	6.67 (5.38–9.11)*
Ethyl acetate fraction ( $n = 7$ )	0.8	6.16 (5.01–8.50)*	5.51 (7.25–10.37)*
Butanol fraction ( $n = 6$ )	30	3.83 (2.51–4.71)*	5.91 (4.49–7.61)*
Aqueous fraction ( $n = 6$ )	137	3.54 (2.05–4.56)	5.79 (4.99–8.78)
Hexane fraction ( $n = 6$ )	0.7	5.69 (4.74–6.54)*	5.98 (4.17–7.88)*
Chloroform fraction ( $n = 6$ )	1.2	4.98 (3.01–6.76)*	6.37 (5.15–8.57)*

Isolated rat islets were incubated for 60 min with ethanol extract and five partition fractions of *A. racemosus* (30 µg/ml) in the presence of 3 or 11 mM glucose. Mann–Whitney  $U$ -test was used to evaluate statistical significance. \* $P < 0.05$ , <sup>†</sup> $P < 0.01$  compared with control (3 mM glucose with no extract).



**Figure 2** Effects of (A) ethanol extract and (B) five fractions of *Asparagus racemosus* on insulin release from perfused rat pancreas. Each point is the mean  $\pm$  s.d. of four to five separate experiments. Pancreas was perfused (1 ml/min) with the extract/fraction at a dose of 1 mg/min. The glucose concentrations in the perfusate were raised from 2.8 to 11.1 mM. Abbreviations: Ext, extract; G, glucose; fr, fraction; Hex, hexane; Chlor, chloroform; EtAc, ethyl acetate; Bu, butanol; Aq, aqueous.

**Table 2** Effects of ethanol extract and partition fractions of *A. racemosus* on insulin release from perfused pancreas (peak value). Values are mean  $\pm$  s.d. ( $n=4-6$ )

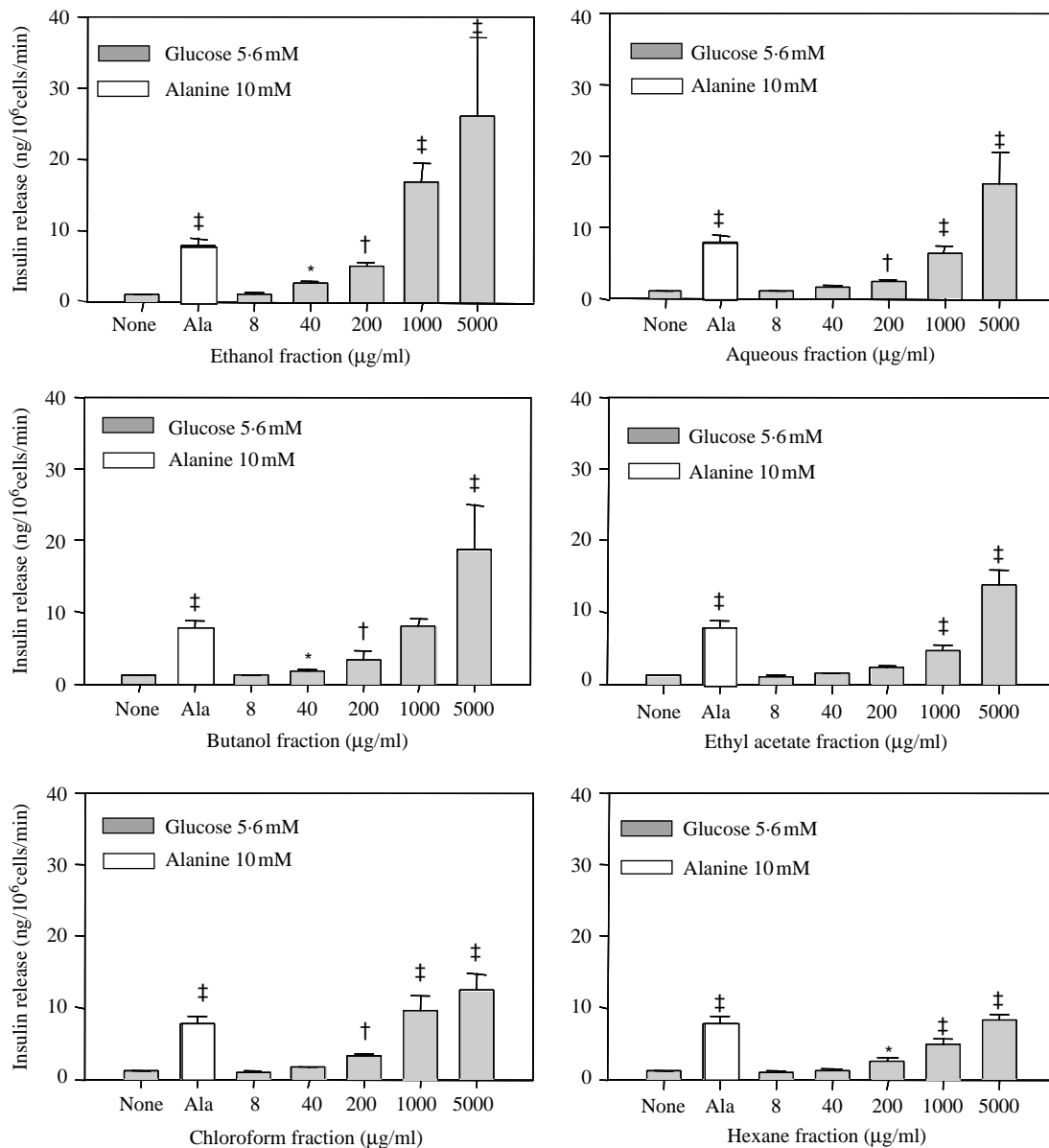
Group	Highest peak of insulin secretion (ng/ml)			
	Basal 2.8 mM glucose	2.8 mM glucose + extract/fraction	11 mM glucose	11 mM glucose + extract/fractions
Ethanol extract	0.06 $\pm$ 0.005	1.27 $\pm$ 0.09 <sup>†</sup>	1.29 $\pm$ 0.13	1.45 $\pm$ 0.10 <sup>‡</sup>
Aqueous fraction	0.05 $\pm$ 0.004	0.62 $\pm$ 0.08 <sup>†</sup>	0.91 $\pm$ 0.09	0.99 $\pm$ 0.79
Butanol fraction	0.06 $\pm$ 0.009	0.18 $\pm$ 0.09*	0.79 $\pm$ 0.08	0.66 $\pm$ 0.09
Ethylacetate fraction	0.04 $\pm$ 0.008	1.16 $\pm$ 0.12 <sup>†</sup>	1.31 $\pm$ 0.11	1.42 $\pm$ 0.13 <sup>‡</sup>
Chloroform fraction	0.05 $\pm$ 0.006	0.91 $\pm$ 0.08 <sup>†</sup>	1.21 $\pm$ 0.10	1.31 $\pm$ 0.12 <sup>‡</sup>
Hexane fraction	0.03 $\pm$ 0.004	1.22 $\pm$ 0.15 <sup>†</sup>	1.22 $\pm$ 0.95	1.34 $\pm$ 0.11 <sup>‡</sup>

\* $P < 0.01$ , <sup>†</sup> $P < 0.001$  compared with 2.8 mM glucose. <sup>‡</sup> $P < 0.05$  compared with 11 mM glucose.

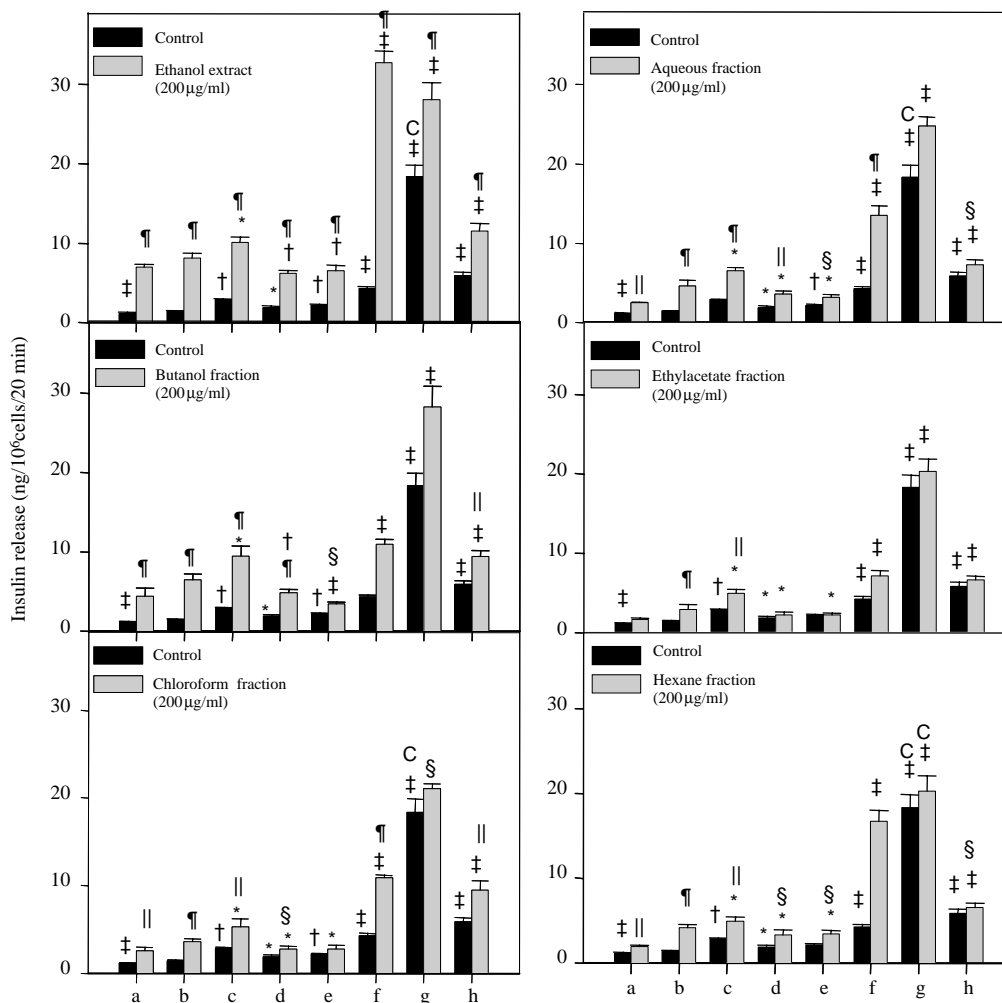
1.2 ± 0.1 ng/ml). These fractions stimulated further insulin secretion at 11.1 mM glucose (peak output, 1.2 ± 0.9 vs 1.3 ± 0.1 ng/ml, 1.3 ± 0.1 and 1.4 ± 0.1 ng/ml respectively; *P* < 0.05). Aqueous and butanol fractions showed less prominent effects on insulin release, especially at the lower glucose concentration 0.05 ± 0.004 ng/ml (2.8 mM glucose (basal)) vs 0.91 ± 0.09 ng/ml (aqueous extract) and 0.06 ± 0.01 ng/ml (basal) vs 0.79 ± 0.08 ng/ml (butanol extract respectively; Fig. 2B and Table 2).

*Effects of A. racemosus on insulin secretion from isolated islets*

Ethanol extract and four partition fractions (ethyl acetate, butanol, chloroform and hexane) induced a significant increase in insulin secretion from isolated rat islets compared with 3 mM glucose (*P* < 0.01, < 0.01, and < 0.05 respectively; Table 1). Increasing glucose concentrations from 3 to 11.1 mM caused a two-fold increase in insulin release. The effects of stimulatory plant extract/fractions were also



**Figure 3** Acute effects of different concentrations of ethanol extract and five partition fractions of *Asparagus racemosus* on insulin release from BRIN-BD11 cells. Results are the mean ± s.d. of eight separate observations. \**P* < 0.05, †*P* < 0.01, ‡*P* < 0.001 compared with 5.6 mM glucose. One-way ANOVA was performed and pairwise comparisons to the control (5.6 mM glucose) group used Dunnett's test to preserve an overall error rate of 5%. Ala, alanine.



**Figure 4** Effects of ethanol extract and five partition fractions of *A. racemosus* on insulin release from BRIN-BD11 cells in the presence of established stimulants and inhibitors of insulin secretion. Results are the mean  $\pm$  s.d. of eight separate observations. \* $P < 0.05$ , † $P < 0.01$ , ‡ $P < 0.001$  compared with respective 5.6 mM glucose (control) in the presence or absence of plant extract. § $P < 0.05$ , ¶ $P < 0.01$ , ¶¶ $P < 0.001$  compared with the respective incubations in the absence of plant extract. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$  compared with respective 16.7 mM glucose (controls) in the presence or absence of plant extract. One-way ANOVA was performed and pairwise comparisons to the control group (5.6 mM glucose) performed using Dunnett's test to preserve an overall error rate of 5%. Letter codes: a, 0 mM glucose; b, 5.6 mM glucose; c, 5.6 mM glucose + 50  $\mu$ M verapamil; d, 16.7 mM glucose; e, 5.6 mM glucose + 300  $\mu$ M diazoxide; f, 5.6 mM glucose + 100  $\mu$ M IBMX; g, 16.7 mM glucose + 30 mM KCl; h, 5.6 mM glucose + 200 mM tolbutamide.

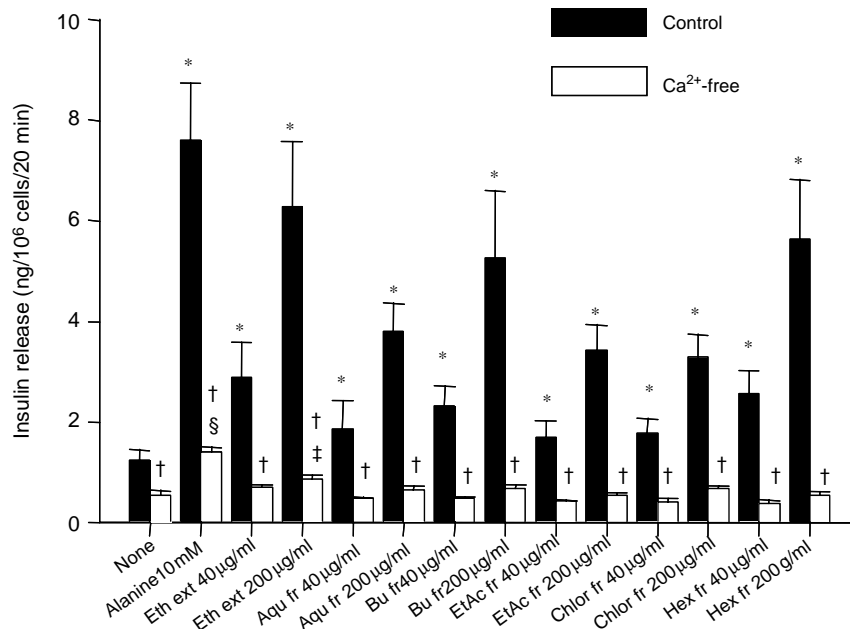
enhanced. The aqueous fractions did not show any stimulatory effects on insulin release in these experiments.

#### *Effects of A. racemosus on insulin secretion from clonal BRIN-BD11 cell-line*

Figure 3 shows the effects of a range of concentrations of ethanol extract and five partition fractions (aqueous, butanol, ethyl acetate, chloroform and hexane) of *A. racemosus* on insulin secretion from BRIN-BD11 cells. Alanine (10 mM) was used as a positive control in this set of experiments. Ethanol extract (40–5000  $\mu$ g/ml) stimulated insulin release in

a concentration-dependent manner compared with control (5.6 mM glucose). The higher concentrations (1000–5000  $\mu$ g/ml) were associated with a 20–30% reduction in cellular viability. Aqueous and chloroform fractions were equipotent at stimulating insulin secretion from 40  $\mu$ g/ml. However, enhanced insulin release was noted at concentrations of 200  $\mu$ g/ml and above with butanol, ethyl acetate and hexane fractions ( $P < 0.05$ –0.001). Concentrations of all five fractions between 40 and 200  $\mu$ g/ml did not affect the cells viability (data not shown).

Further studies using non-toxic concentrations evaluated possible mechanisms underlying the actions of *A. racemosus*.



**Figure 5** Effects of various concentrations of the ethanol extract and partition fractions of *A. racemosus* on insulin release from BRIN-BD11 cells in the presence and the absence of Ca<sup>2+</sup>. Results are mean  $\pm$  s.d. of eight separate observations. \* $P < 0.001$  compared with 5.6 mM glucose in the presence of Ca<sup>2+</sup>. † $P < 0.001$  compared with the respective concentration in the presence of Ca<sup>2+</sup>. ‡ $P < 0.05$ . § $P < 0.001$  compared with 5.6 mM glucose in the absence of Ca<sup>2+</sup>. One-way ANOVA was performed and pairwise comparisons to the control (5.6 mM glucose) group performed using Dunnett's test to preserve an overall error rate of 5%. Abbreviations: Eth ext, ethanol extract; Aqu fr, aqueous fraction; Bu fr, butanol fraction; EtAc fr, ethyl acetate fraction; Chlor fr, chloroform fraction; Hex fr, hexane fraction.

As shown in Fig. 4, ethanol extract of *A. racemosus* (200 µg/ml) exhibited a ninefold increase in insulin release ( $P < 0.001$ ). This effect was significantly enhanced in the presence of 16.7 mM glucose ( $P < 0.05$ ), IBMX ( $P < 0.001$ ) and tolbutamide ( $P < 0.001$ ). Modest inhibitory effects were observed in the presence of diazoxide ( $P < 0.01$ ) and verapamil ( $P < 0.01$ ). The ethanol extract also maintained its ability to increase insulin secretion in cells depolarized with 30 mM KCl (Fig. 3). Similar effects were noted for aqueous, butanol, ethyl acetate, chloroform and hexane fractions in the presence of insulin secretagogues (glucose, IBMX, tolbutamide), inhibitors (diazoxide and verapamil) and depolarizing concentrations of KCl. As shown in Fig. 5, omission of Ca<sup>2+</sup> from incubation buffer significantly reduced ( $P < 0.001$ ) the insulin-releasing ability of the extract and abolished that of the all partition fractions ( $P < 0.001$ ).

#### Effects of *A. racemosus* on intracellular calcium in clonal BRIN-BD11 cell line

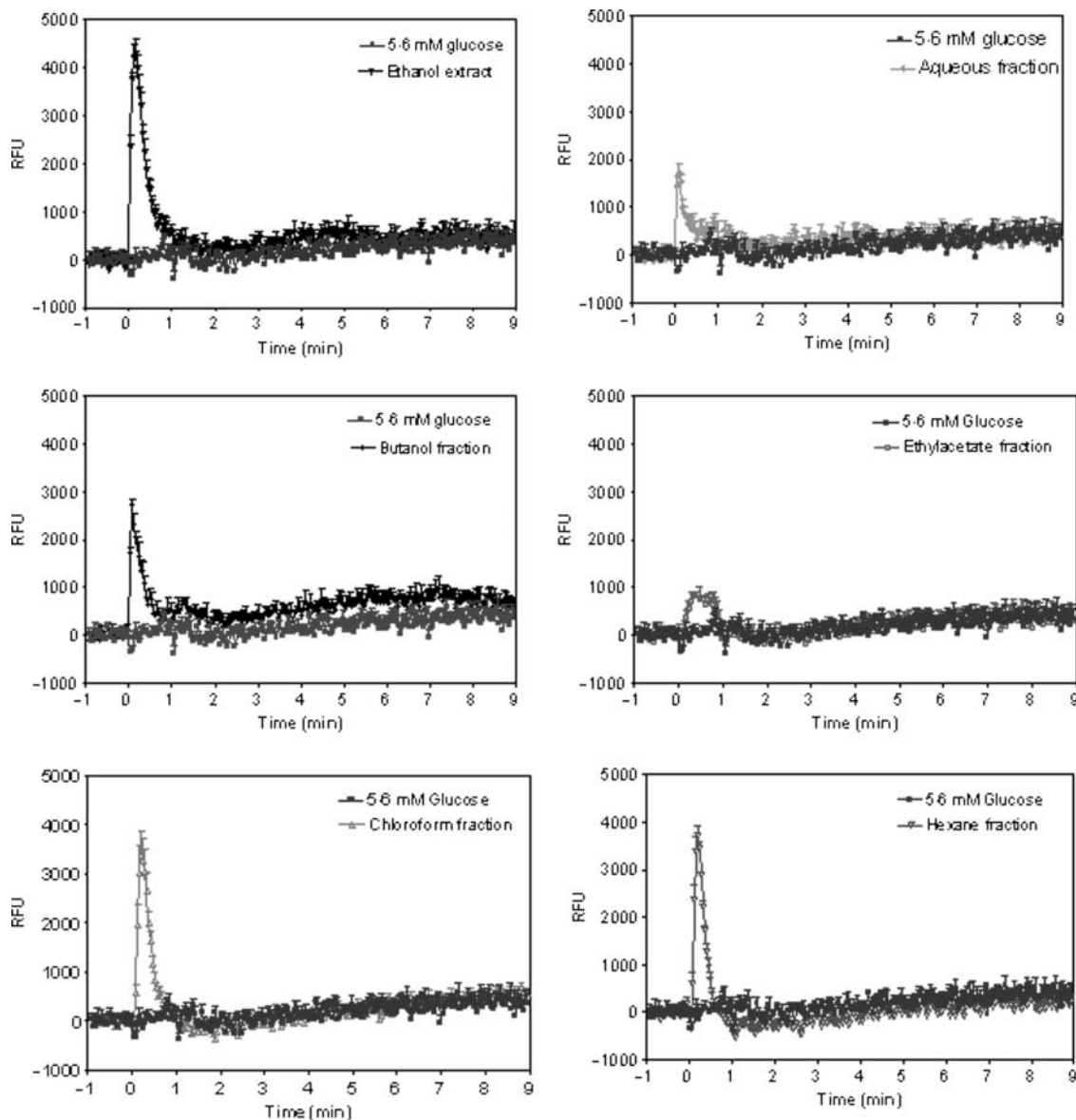
Figure 6 shows the effects of the ethanol extract and five partition fractions (aqueous, butanol, ethyl acetate, chloroform and hexane) on intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>). A sharp increase in ([Ca<sup>2+</sup>]<sub>i</sub>) was observed compared with 5.6 mM

glucose alone. The magnitude of the effect was somewhat less with the aqueous and ethyl acetate fractions.

## Discussion

Presently available drug regimens for the management of diabetes mellitus have certain drawbacks and are not readily available in developing parts of the world (Yudkin 2000, Yach *et al.* 2004). There is a need for a move widely applicable, safer and more effective anti-diabetic drug therapy. In this study, the insulinotropic effects of ethanol extract and five partition fractions (aqueous, butanol, ethyl acetate, chloroform and hexane) of *A. racemosus* were assessed using perfused rat pancreas, isolated rat islets and a clonal rat insulin secreting cell line.

The ethanol extract and three fractions (ethyl acetate, butanol and aqueous) were found to be highly effective in stimulating insulin secretion from the perfused pancreas. This effect was immediate and enhanced further into a biphasic profile at 11.1 mM glucose. Thus, the profile of insulin release from perfused pancreas suggests that the actions of ethanol extract and other fractions might not be limited to first-phase insulin secretion only. Similar effects were



**Figure 6** Effects of ethanol extract and five partition fractions of *A. racemosus* on intracellular  $\text{Ca}^{2+}$  influx in BRIN-BD11 cells. Each data point represents the mean  $\pm$  s.d. for six separate observations. RFU, relative fluorescent unit.

observed in acute studies of insulin release with isolated rat islets. Aqueous fraction had lesser effects and these results suggest that non-polar fractions of *A. racemosus* were more effective in enhancing insulin secretion. Insulinotropic effects of the extract/fractions were also evaluated in BRIN-BD11 cells. Using this cell line, the ethanol extract and each of the five fractions showed stepwise concentration-dependent stimulatory effects on insulin secretion at basal glucose concentration. A clear division was observed between insulin secretion and possible detrimental effects on cell viability. It is noteworthy that small differences in secretory responses were observed with the various  $\beta$ -cell models. However, this is

unsurprising given differences in origin of  $\beta$ -cells, mode of presentation of extract/fraction constituents, heterogeneity and likely concentration dependency.

Further studies to examine the mechanism underlying the actions of *A. racemosus* extracts and fractions indicated that the insulin-releasing machinery of the  $\beta$ -cells has been mediated through specific secretory pathways. For example, the  $\text{K}_{\text{ATP}}$  channel opener, diazoxide (Henquin *et al.* 1992) reduced the insulin-releasing action indicating that part of *A. racemosus* action involves an inhibitory effect on  $\text{K}_{\text{ATP}}$  channels. The voltage-dependent calcium channel blocker, verapamil also significantly reduced insulin release providing evidence



that  $\text{Ca}^{2+}$  plays a key role in mediating the effect. Consistent with this view, insulin secretory effects of the extract were abolished in the absence of extracellular  $\text{Ca}^{2+}$  and the extract provoked immediate elevation of  $[\text{Ca}^{2+}]_i$  in BRIN-BD11 cells. The action of the extract was potentiated by 16.7 mM glucose, suggesting that  $\beta$ -cell glucose metabolism is important for augmentation of insulinotropic effect. However, the extract induced insulin release in cells depolarized by 30 mM KCl or tolbutamide suggesting that depolarization is not the sole mechanism through which the extract exerts its effects. IBMX, an inhibitor of cyclic AMP phosphodiesterase, potentiated insulin release by increased levels of intracellular cAMP (Sharp 1979). Interestingly, effect of IBMX was enhanced by the presence of the extract, suggesting the possible involvement in cAMP production pathway.

The five partition fractions (aqueous, butanol, ethyl acetate chloroform and hexane) of *A. racemosus* showed similar effects on insulin secretion to the parent ethanol extract to glucose, IBMX, tolbutamide and depolarising conditions, diazoxide, verapamil, and  $\text{Ca}^{2+}$  depletion. Similarly, stimulatory effects on intracellular  $\text{Ca}^{2+}$  were observed with the fractions suggesting same or similar active molecules acting through common pathways.

In conclusion, this study has shown that *A. racemosus* extracts exerted significant stimulatory effects on insulin secretion mediated through physiological pathways. Such an action may clearly contribute to the observed anti-hyperglycaemic actions reputed in rats and rabbits (Rana *et al.* 1994). Future work directed towards the identification of active principle(s) from *A. racemosus* may provide the opportunity for the development of a novel class of agents for the treatment of diabetes.

## Acknowledgements

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 26 July 2006

Accepted 10 October 2006