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## Evaluation of the Insulinotropic Activity of Malaysian Traditional Plants Extract

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**Abstract:** In this study, methanolic extracts of 14 traditional plants in Malaysia were screened for insulinotropic properties, using rat pancreatic  $\beta$ -cell lines, BRIN-BD11 cells. In 30 min acute static incubation test, all 14 plants showed varying degree of responsiveness in insulin release with *Labisa pumila*, *Morinda citrifolia*, *Momordica charantia* and *Tinospora crispa* having high insulinotropic activities. These plants also displayed appreciably low cytotoxic activities. These results show a promising avenue for development of novel insulin secretagogues.

**Key words:** Insulinotropic, malaysian traditional plants, rat pancreatic  $\beta$ -cell lines

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### INTRODUCTION

Insulinotropic drugs have been used as oral hypoglycemic agents which stimulate insulin secretion by pancreatic  $\beta$ -cells in the treatment of Non-Insulin Dependent Diabetes Mellitus (NIDDM) for almost 45 years. These drugs, namely tolbutamide, glibenclamide and glibepiride trigger insulin secretion primarily through binding to the sulphonylurea receptor subunit on the  $\beta$ -cell membrane (Sheehan, 2003; Rendell, 2004). The possibility of exploiting the rat pancreatic cell lines to study the insulinotropic effects is currently considered with much enthusiasm in developing new anti-diabetic agents (Mathews *et al.*, 2006; McClenaghan, 2007). Considerable potential is also afforded for rapid screening and isolation of novel anti-diabetic entities from plants and other natural resources based on insulinotropic activities using pancreatic  $\beta$ -cell lines.

Many traditional plants have been used as a source for drugs development and about 800 plants have been reported to possibly possess anti-diabetic potential (Alarcon-Aguilara *et al.*, 1998). Even the discovery of the widely used hypoglycemic drug, metformin came from the traditional plant known as *Galega officinalis* (Grover *et al.*, 2002). Thus, plants are a remarkably

potential source of anti-diabetic drugs. However, this fact has not gained enough momentum in the scientific community due to several reasons; one of which is the lack of belief among the practitioners of conventional medicine over herbal remedies. On the other hand, there are not many scientific data to verify and validate the usage of alternative medicine.

Hence, these reasons have prompted the present study in which *in vitro* evaluation of insulinotropic activities of methanolic extract of 14 Malaysian traditional plants using the rat pancreatic  $\beta$ -cell lines, BRIN-BD11 cells has been carried out. A plant-screening for potential antidiabetic was based on the ethnomedical knowledge of the local population. Insulin secretion was assessed using BRIN-BD11 cells, produced by electrofusion of immortal RINm5F cells with New England Deaconess Hospital rat pancreatic  $\beta$ -cells (McClenaghan *et al.*, 1996). Secretory characteristics of this cell line had been described intensively and widely used as *in vitro* model for insulin secretory studies (Beauwens *et al.*, 2006; Kaminski *et al.*, 2004; Zhang *et al.*, 2004). The cytotoxicity effect of all 14 plant extracts on the rat pancreatic  $\beta$ -cell lines, BRIN-BD11 cells was also examined using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay.

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## MATERIALS AND METHODS

**Plant materials:** Plant materials were collected from the Forest Research Institute Malaysia (FRIM) and Phytomedicine Laboratory, Institute of Bioscience, Universiti Putra Malaysia. All plants as shown in Table 1 were authenticated by botanist and voucher specimens were deposited in the herbarium at the Laboratory of Phytomedicine, Institute of Bioscience, Universiti Putra Malaysia. Fresh plants sample were cut into smaller pieces and macerated in methanol for 5 days. Each sample was extracted twice. The methanolic extract was then concentrated under reduced pressure at 40°C and stored at -20°C until ready to be tested.

**Insulin secretion experiments:** Insulin secretion activity was carried out according to Gray and Flatt, (1998) with slight modifications. BRIN-BD11 cells were seeded at concentration of  $2.5 \times 10^5$  cells well<sup>-1</sup> in a 24 well plate cultured in 1 mL of RPMI 1640 culture media containing 11.1 mM of glucose supplemented with 10% (v/v) of Foetal Bovine Serum (FBS) and antibiotics (100 IU mL<sup>-1</sup> penicillin and 0.1 g L<sup>-1</sup> streptomycin) to allow attachment overnight prior to acute tests. Cells were washed thrice with Krebs Ringer Bicarbonate buffer (KRBB, pH 7.4) supplemented with 0.5% (w/v) Bovine Serum Albumin (BSA) and 1.1 mM of glucose and preincubated for 40 min at 37°C. The buffer was removed and the cells were then incubated for 30 min with 1 mL KRBB test buffer supplemented with 0.5% (w/v) bovine serum albumin and 1.1 mM of glucose in the presence of plant extract. Plant extract were added and final concentrations were adjusted ranging from 0.1, 0.5, 1 and 5 mg mL<sup>-1</sup>. Insulinotropic antidiabetic drugs, Glybenclamide and Tolbutamide were used as positive control drugs meanwhile cells with 1 mL KRBB test buffer supplemented with 0.5% (w/v) bovine serum albumin and 1.1 mM of glucose in the absence of plant extract were used as control for basal insulin secretion. Following incubation, aliquots were removed from each well and stored at -20°C until it was assayed for insulin.

**Insulin assay:** The insulin level secreted by BRIN-BD11 cells was measured by an enzyme-linked immunosorbent assay using a commercial rat insulin ELISA (DRG Instruments GmbH, Germany) and rat insulin was used as a standard. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on insulin molecule. During incubation, insulin in the sample reacts with anti-insulin antibodies bound to the microtiter wells and peroxidase conjugated anti-insulin antibodies. A simple

washing step removes unbound enzyme labelled antibody. The bound conjugate was detected by reacting with 3,3',5,5'-tetramethylbenzidine (TMB) and read with the BioRad Reader at 450 nm (reference filter 650 nm).

**Cytotoxicity assay:** The cytotoxic effects of the plant extracts on pancreatic  $\beta$ -cell line were determined using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay (Cetin and Bullerman, 2005). BRIN-BD11 cells were seeded in 96-well plate at a concentration of  $1 \times 10^5$  cells per well. The plate was incubated for 24 h at 37°C in an atmosphere containing 95% air and 5% CO<sub>2</sub>, allowing the cells to attach to the bottom of the wells. The next day, the cells were treated with different concentrations of methanolic plant extracts for 72 h. A volume of 20  $\mu$ L of MTT solution (5 mg mL<sup>-1</sup>) was added to each wells and incubated for 4 hour at 37°C. The medium was aspirated from the well and then 200  $\mu$ L dimethyl sulfoxide (DMSO) was added into each well to solubilize formazan. The plate was agitated on a plate shaker for 5 min and absorbance was measured at 570 nm with ELISA Reader. Dose-response curves were computer-plotted after converting the mean data values to percentages of the control response. The half maximal inhibition concentration (IC<sub>50</sub>) was evaluated from the dose-response curve.

## RESULTS AND DISCUSSIONS

Two sulphonylurea drugs were selected as the positive control drugs to investigate the insulinotropic activity on rat pancreatic  $\beta$ -cell, BRIN-BD11 cell culture. Both drugs gave almost similar pattern of secretory responses of BRIN-BD11 cells. Glybenclamide and Tolbutamide at 1 mM increased insulin release by more than 3-fold compared with 1.1 mM glucose alone as control (data not shown).

*In vitro* insulinotropic evaluation of 14 Malaysian traditional plants is shown in Table 1. In this study, four concentrations of the plant extracts (0.1, 0.5, 1.0 and 2.0 mg mL<sup>-1</sup>) were used. Each plant extract was able to show insulin secretory response although there were obvious differences in terms of both magnitude and pattern of insulin secretory responsiveness.

The insulin secretion study on *Andropogon paniculata* showed maximum potential activity 2-3 fold at 2.0 mg mL<sup>-1</sup> when compared with 1.1 mM glucose alone. The action of *Gynura sarmientosa*, *Gynura procumbens*, *Preskio bleo*, *Scaphoclamys* sp., *Taca integrifolia* and *Zinger officinale* extracts only shows 1-2 fold insulin stimulatory effect when compared with basal insulin release recorded at 1.1 mM glucose alone. It was also

Table 1: Potentiating insulin-secretory activity of BRIN-BD11 treated with methanolic extracts of Malaysian traditional plants

Plant and part used	Potentiating activity concentration (mg mL <sup>-1</sup> )			
	0.1	0.5	1.0	2.0
<i>Andrographis paniculata</i> (leaves)	+	+	+	++
<i>Ficus deltoidea</i> (leaves)	++	+	+	+
<i>Gynura sarmetosa</i> (leaves)	+	+	+	+
<i>Gynura procumbens</i> (leaves)	+	++	+	+
<i>Labisa pumila</i> (leaves)	+	++	+++	+++
<i>Momordica charantia</i> (fruits)	++	+++	+++	+++
<i>Morinda citrifolia</i> (fruits)	+	+	+++	+++
<i>Orthosipon spicatus</i> (leaves)	++	++	++	++
<i>Preskia bleo</i> (leaves)	+	+	+	-
<i>Scaphoclamys</i> sp. (leaves)	+	+	+	+
<i>Strobilanthus crispus</i> (leaves)	-	-	-	-
<i>Tacca integrifolia</i> (leaves)	+	+	-	-
<i>Tinospora crispa</i> (stem bark)	++	++	++	+++
<i>Zingiber officinale</i> (leaves)	+	+	+	+

Potentiating of the insulin-secretory response with plants extract. Maximum potentiating activity was: -: No activity; +: 1-2 fold; ++: 2-3 fold; +++: More than 3-fold insulin stimulatory effect compared with 1.1 mM glucose alone (control)

noteworthy that the *Orthosipon spicatus* extract showed no increment in insulin-secretory response throughout various test concentrations. However, *Ficus deltoidea* showed some inhibitory action on insulin secretion at higher concentrations. This may be explained in regard to the cytotoxicity effect or presence of substances that inhibit insulin secretion in this extract (Table 1).

The responsiveness of BRIN-BD11 cells to *Labisa pumila* and *Morinda citrifolia* extract showed promising stimulatory effect in a dose-dependent manner and secreted more than 3-fold insulin releases at 1.0 mg mL<sup>-1</sup> when compared with control. *Tinospora crispa* also showed almost similar pattern of insulin-stimulatory response with more than 3-fold insulin release at 2.0 mg mL<sup>-1</sup>. However, methanolic extract of *Momordica charantia* fruits gave the greatest insulin stimulatory effect in all the 14 plants extract tested. BRIN-BD11 cells incubated with 0.5 mg mL<sup>-1</sup> of *Momordica charantia* fruits methanolic extract resulted in more than 3-fold stimulation of insulin release compared with 1.1 mM glucose alone. This result is in agreement with that reported by Yibchok-Anun *et al.* (2006) that an extract from the fruits of *Momordica charantia* appears to enhance secretion of endogenous insulin. Similar effect was also reported for the *Tinospora crispa* (Noor and Ashcroft, 1998). In contrast, *Strobilanthus crispus* extract showed no stimulatory effect on insulin secretion at all concentrations.

In order to determine whether cytotoxicity of the extract affect insulinotropic activity, IC<sub>50</sub> value of all plants extract were examined. The respective IC<sub>50</sub> values for all the extracts showed that cytotoxicity had an impact on insulin- stimulatory response. Methanolic extract with

Table 2: Inhibition concentration 50% (IC<sub>50</sub>) of Malaysians traditional plants and sulphonylureas drugs (Tolbutamide and Glybenclamide) on BRIN-BD11 cell lines by MTT assay

Plants sample	IC <sub>50</sub> value (µg mL <sup>-1</sup> )
<i>Andrographis paniculata</i>	90.00
<i>Ficus deltoidea</i>	125.00
<i>Gynura sarmetosa</i>	375.00
<i>Gynura procumbens</i>	300.00
<i>Labisa pumila</i>	175.00
<i>Momordica charantia</i>	953.00
<i>Morinda citrifolia</i>	2500.00
<i>Orthosipon spicatus</i>	180.00
<i>Preskia bleo</i>	98.00
<i>Scaphoclamys</i> sp.	345.00
<i>Strobilanthus crispus</i>	4240.00
<i>Tacca integrifolia</i>	212.00
<i>Tinospora crispa</i>	800.00
<i>Zingiber officinale</i>	85.00
Tolbutamide	0.17 mM
Glybenclamide	0.20 mM

low cytotoxicity level showed higher insulin secretion activity, particularly extract from *Labisa pumila*, *Morinda citrifolia* and *Tinospora crispa* (Table 2).

## CONCLUSION

The present study shows that methanolic extracts of a range of Malaysian traditional plants exhibit interesting insulinotropic properties *in vitro*. The potential shown by certain plants extract especially *Labisa pumila*, *Morinda citrifolia*, *Momordica charantia* and *Tinospora crispa* offer an exciting preliminary study opportunity in the search for novel antidiabetic agents in other Malaysian traditional plants at the same time extending a whole new horizon of botanical medicine.

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## REFERENCES

- Alarcon-Aguilara, F.J., R. Roman-Ramos, S. Perez-Gutierrez, A. Aguilar-Contreras, C.C. Contreras-Weber and J.L. Flores-Saenz, 1998. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J. Ethnopharmacol., 61: 101-110.
- Beauwens, R., L. Best, N. Markadieu, R. Crutzen, K. Louchami, P. Brown, A.P. Yates, W.J. Malaisse and A. Sener, 2006. Stimulus-secretion coupling of hypotonicity-induced insulin release in BRIN-BD11 cells. Endocrine, 30: 353-364.

- Cetin, Y. and L.B. Bullerman, 2005. Cytotoxicity of Fusarium mycotoxins to mammalian cell cultures as determined by the MTT bioassay. *Food Chem. Toxicol.*, 43: 755-764.
- Gray, A.M. and P.R. Flatt, 1998. Actions of the traditional anti-diabetic plant, *Agrimony eupatoria* (agrimony): Effects on hyperglycaemia, cellular glucose metabolism and insulin secretion. *Br. J. Nutr.*, 80: 109-114.
- Grover, J.K., S. Yadav and V. Vats, 2002. Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol.*, 81: 81-100.
- Kaminski, A., H. Gao and N.G. Morgan, 2004. Involvement of the cGMP signalling pathway in the regulation of viability in insulin-secreting BRIN-BD11 cells. *FEBS Lett.*, 559: 118-124.
- Mathews, J.N., P.R. Flatt and Y.H. Abdel-Wahab, 2006. *Asparagus adscendens* (Shweta musali) stimulates insulin secretion, insulin action and inhibits starch digestion. *Br. J. Nutr.*, 95: 576-581.
- McClenaghan, N.H., C.R. Barnett, E. Ah-sing, Y.H.A. AbdelWahab, F.P.M.O. Harte, T.W. Yoon, S.K. Swanston-Flatt and P.R. Flatt, 1996. Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. *Diabetes*, 45: 1132-1140.
- McClenaghan, N.H., 2007. Physiological regulation of the pancreatic beta-cell: Functional insights for understanding and therapy of diabetes. *Exp. Physiol.*, 92: 481-496.
- Noor, H. and S.J. Ashcroft, 1998. Pharmacological characterisation of the antihyperglycaemic properties of *Tinospora crispa* extract. *J. Ethnopharmacol.*, 62: 7-13.
- Rendell, M., 2004. The role of sulfonylureas in the management of type 2 diabetes. *Drugs*, 64: 1339-1358.
- Sheehan, M.T., 2003. Current therapeutic options in type 2 Diabetes Mellitus: A practical approach. *Clin. Med. Res.*, 1: 189-200.
- Yibchok-Anun, S., S. Adisakwattana, C.Y. Yao, P. Sangvanch, S. Roengsumran and W.H. Hsu, 2006. Slow acting protein extract from fruit pulp of *Momordica charantia* with insulin secretagogue and insulinomimetic activities. *Biol. Pharm. Bull.*, 29: 1126-1131.
- Zhang, Q., M. Kohler, S.N. Yang, F. Zhang, O. Larsson and P.O. Berggren, 2004. Growth hormone promotes  $Ca^{2+}$ -induced  $Ca^{2+}$  release in insulin-secreting cells by ryanodine receptor tyrosine phosphorylation. *Mol. Endocrinol.*, 18: 1658-1669.