The Traditional Plant, *Andrographis paniculata* (Sambiloto), Exhibits Insulin-Releasing Actions in Vitro

Aris Wibudi*, Bambang Kiranadi**, Wasmen Manalu**, Adi winarto**, Slamet Suyono***

ABSTRACT

Aim: *to examine the effect of A.paniculata on pancreatic β-cells.*

Methods: *sixty minutes incubation of BRIN-BD11 in Modified Kreb-Ringer Solution containing 16.7 mM glucose (KRB-3) + 0.625 – 2.5 mg/mL A.paniculata evoked 1.7 – 3.73 fold of insulin secretion compared to 16.7 mM glucose only (p = 0.003 – p < 0.001).*

Results: *compared to the effect of 100 μM glibenclamide, 60 minutes incubation of BRIN-BD11 in KRB-3 containing 1.25 and 2.5 mg/mL A. paniculata evoked 1.5 fold (p=0.034) and 2.3 fold (p=0.001) insulin secretion. Twenty minutes incubation of BRIN-BD11 in KRB-3 + 0.625-5 mg/mL A.paniculata, evoked 1.4 – 4.7 fold (p = 0.002 – p < 0.001) of insulin secretion compared to 16.7 mM glucose only. Twenty minutes incubation of BRIN-BD11 in KRB-1 containing 1.11 mM glucose + 0.625 – 10 mg/mL A.paniculata, evoked 1.3 – 3.7 fold (p = 0.019 – p < 0.001) of insulin secretion compared to 16.7 mM glucose only.*

Conclusion: *this study assumed that A.paniculata was a very strong, dose dependent insulinotropic agent, glucose dependent and independent insulin secreting agent. This study also assumed that A.paniculata affected one of the membrane receptors, mostly ATP-dependent potassium channels (K^+_{ATP}).*

Key words: *A. paniculata, insulin secretion cell line, mechanism of action in vitro.*

INTRODUCTION

It is very common to use traditional anti-diabetic plant or herbal alone or in combination with oral anti diabetic agent to achieve better blood glucose control in Indonesia, especially in rural area. Bratawali (*Tinospora crispa*) and Sambiloto (*Andrographis paniculata*) are the most common traditional plants or herbal that are used to achieve lower blood glucose in diabetes, even though the mechanism has not yet been defined. *A.paniculata* has been reported to have a number of medicinal properties including the ability to reduce allergic reaction[1], treat uncomplicated upper respiratory infection[2], anti-microbial[3,4], anti-malaria[5], immuno-modulator[6,7], and lowered blood glucose among Streptozotocin-diabetic rat[8-12] and Aloxan-diabetic rat.[13] As an anti-diabetic, the effect of *A.paniculata* was presumed to have affected the extra-pancreatic organ such as: inhibition of glucose absorption from gut[13], improved intra-cellular glucose metabolism[8], and improved muscle-glucose uptake by improving GLUT-4-mRNA transcription.[10]

It is strongly recommended to know the mechanism of action whether oral anti-diabetic drug (OAD) or herbal, and not only its blood glucose lowering effect, thus they can be classified into one of the OAD groups such as: a). Reduction of hepatic glucose production (HGP), b). Insulin secreting agent or insulin secretagogue, c). Inhibitor of glucose absorption from gut, or d). Insulin sensitizer that can improve insulin resistance in type-2 DM. It is intended to avoid any selection of the same group as a combination. Up to now the effect of *A. paniculata* towards pancreatic β-cells has not been established, so the aim of this study is to examine the insulinotropic effect of *A. paniculata* towards BRIN-BD11.

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METHODS

Andrographis paniculata

Andrographis paniculata nees (*A.paniculata*), number suksesi Cimangu, was obtained from Balai Penelitian Tanaman Rempah dan Obat (BALITTRO), Bogor, dried for 3 days, in an incubator at 40^0 C, homogenized to a fine powder, and stored in a vacuum container at room temperature until use. Stock solution or concentrate of 40 mg/mL *A.paniculata* was made freshly, by pouring 4 gr AP powder into 100 mL boiled (100^0 C) aquabidest, stirred for 15 minutes, reconstituted or readjusted the volume with aquabidest to achieve 100 mL, and then centrifugated for 15 minutes at 2000 rpm. The solution was separated from the solid matter, and used as one of the Modified Kreb-Ringer Solution component, depending on the research plan.

Insulin Secretion in Vitro

A glucose-responsive clonal insulin-secreting cell line BRIN-BD11 (kindly provided by Prof.Dr.dr.Andrè Herchuelz, Brussels, Belgium), produced by electrofusion of immortal RINm5F cell with New England Deaconess Hospital rat pancreatic β-cell, was used to evaluate insulin secretion.[14-21] The appropriateness of BRIN-BD11 cells for screening of antidiabetic plant materials and characterization of novel insulin-releasing natural products have been described elsewhere.[22-28] Insulin secretion of BRIN-BD11 as insulinotropic response was measured after 60 and 20 minutes incubation in the media Modified Kreb-Ringer solution. BRIN-BD11 was seeded at concentration of 0.3 X 10^6 cells/well in 24-well plates (Costar, USA), cultured in RPMI 1640 that is fortified with 2 mM L-glutamin, containing 11.1 mM Glucose, 10% Foetal Bovine Serum, and antibiotic Penicillin 100 U/mL – Streptomycin100 µg/mL to allow attachment overnight prior to acute tests, in an incubator at 37^0 C and 5% CO_2. Cells were washed thrice with Modified Kreb's-Ringer buffer solution-1= KRB-1 (115 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24 mM $NaHCO_3$, 10 mM HEPES free acid, 1g/L Bovine Serum Albumin = BSA, 1.11 mM glucose, 15 minutes gassed with 5% CO_2, pH 7.4), and preincubated for 40 minutes at 37^0 C. Unless otherwise stated, cells were then incubated for 60 and 20 minutes with 1 ml KRB-1 or 3 (containing 16.7 mM glucose) in the absence and presence of plant extract, diazoxide (an established opener of K^+_{ATP} channels) and other test agents. Following incubation, aliquots were removed from each well, centrifugated for 5 minutes at 1500 rpm to separate the aliquot from the detached cells, for insulin assay. The method of insulin assay was direct sandwich ELISA, with commercial insulin kit Mercodia Rat Insulin ELISA. Data were evaluated using Student's unpaired *t*-test. Groups were considered to be significantly different if $p<0.05$.

RESULTS

The number of BRIN-BD11 doubled, 6.10^5 in average per well after 24 hours or overnight pre-incubation. Simple water or aqueous extract or simple infusion of *A. paniculata* had a stimulatory effect on insulin secretion by BRIN-BD11 whether at 16.7 mM or 1.11 mM glucose. Sixty minutes incubation of BRIN-BD11 in the media KRB-3, containing 16.7 mM glucose +0.625, 1.25, and 2.5 mg/mL *A. paniculata,* respectively, evoked 1.74 (p=0.003), 2.36 (p=0.001) and 3.7 (p<0.001) fold of insulin secretion, respectively, compared to insulinotropic effect of 16.7 mM glucose (0.529 ± 0.067 ìg/L/6.10^5cells). Similar insulinotropic effect of *A. paniculata* towards BRIN-BD11 was also shown after 20 minutes incubation, 0.625 – 5 mg/mL *A. paniculata* evoked 1.4 (p=0.023), 2.4 (p=0.001), 4 (p=0.001) and 4.7 (p<0.001) fold of insulin secretion, compared to the insulinotropic effect of 16.7 mM glucose towards BRIN-BD11 (0.344 ± 0.058 µg/L/6.10^5cells). (Table 1)

Table 1. Static insulin measurement (µg/L/6.10^5cells) after 60 minutes (A) and 20 minutes incubation (B) of BRIN-BD11 in media KRB-3 containing 16.7 mM glucose + various concentrations of *A. paniculata*

Concentration of *A. Paniculata* (mg/mL)	Insulin secretion (µg/L/6.10^5 cells)	
	A	B
0	0.529 ± 0.067	0.344 ± 0.058
0.625	0.920 ± 0.070	0.486 ± 0.088
1.25	1.250 ± 0.173	0.825 ± 0.125
2.5	1.972 ± 0.042	1.394 ± 0.160
5	0.972 ± 0.133	1.607 ± 0.096
10	0.405 ± 0.064	0.754 ± 0.088

n = 3; 0 = media without *A. paniculata*

Twenty-five, 50, 100 µM glibenclamide and 25 meq KCL, were used as a positive control. In this study, glibenclamide had a dose-dependent stimulatory effect on insulin secretion by BRIN- BD11 (0.584 ± 0.049, 0.672 ± 0.045, and 0.844 ± 0.084 µg/L/6.10^5 cells) respectively, at 16.7 mM glucose. Sixty minutes incubation of BRIN-BD11 in the media containing 25 mEq KCL evoked equal insulin secretion (0.803 ± 0.02 µg/L/6.10^5 cells) compared to the insulinotropic effect of 100 µM glibenclamide. (Table 2)

Table 2 Static insulin measurement (µg/L/6.10^{5}cells) after 60 minutes incubation of BRIN-BD11 in media KRB-3 containing 16.7 mM glucose + various concentration of glibenclamide and 25 meq KCl

Concentration of glibenclamide (µM)	Insulin secretion (µg/L/6.10^5 cells)
25	0.584 ± 10149
50	0.672 ± 0.045
100	0.844 ± 0.084
25 meq KCl	0.803 ± 0.02

n = 3

Insulin secretion as insulinotropic effect of *A. paniculata* towards BRIN-BD11 was also seen after 20 minutes incubation in the media KRB-1 containing 1.11 mM glucose. The insulin secretion increased correspondingly with the increase of *A. paniculata* concentration. The greatest insulin secretion (2.7 fold) was seen at 10 mg/mL *A. paniculata* ($p<0.001$), and the lowest insulinotropic effect was seen (1.3 fold) at 0.625 mg/mL *A. paniculata* ($p = 0.019$), compared to insulinotropic effect of 16.7 mM glucose towards BRIN-BD11 (Table 3).

Table 3. Static insulin measurement (µg/L/6.10^{5}cells), after 20 minutes incubation of BRIN-BD11 in media KRB-1 containing 1.11 mM glucose + various concentrations of *A. paniculata*

Concentration of *A. Paniculata* (mg/mL)	Insulin secretion (mg/L/6.10^5 cells)	*p*
0*	0.344 ± 0.058	
0.625	0.455 ± 0.053	0.019
1.25	0.548 ± 0.051	< 0.001
2.5	0.715 ± 0.065	< 0.001
5	0.847 ± 0.162	< 0.001
10	0.921 ± 0.052	< 0.001

n = 3; 0* = media containing 16.7 mM glucose, without *A. paniculata*

DISCUSSION

This study used simple water or aqueous extraction or simple infusion of *A. paniculata* nees, without striving to get active ingredients such as andrographolides, as done by other researchers.[22-25, 29-32] The other reason being that *A. paniculata* is usually used as a simple water extraction or infusion by boiling the leaves, or even consumed in capsulated form. Thus this study could evaluate directly whether any therapeutic benefit can be obtained from the simple extraction or infusion of *A. paniculata* or not. Study of active ingredient could not reflect the benefit of the herbal.

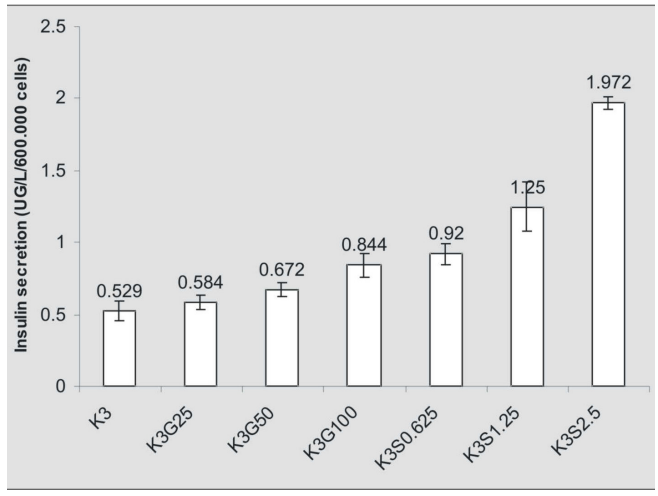

Figure 1. Static insulin measurement after 60 minutes incubation of BRIN-BD11 in media KRB containing 16.7 mM glucose (KRB-3). (K3 = KRB-3; K3K25 = KRB-3+KCl 25meq; K3G25-100 = KRB-3+25-100ìM glibenclamide; K3S0.625-10= KRB-3 + 0.625 -10 mg/mL *A.paniculata*). (n=3)

Sixty minutes incubation of BRIN-BD11 in the media KRB-3 containing 16.7 mM glucose + 0.625, 1.25, and 2.5 mg/mL *A. paniculata,* respectively, evoked 1.74 ($p=0.003$), 2.36 ($p=0.001$), and 3.7 ($p<0.001$) fold of insulin secretion, compared to insulin secretion in KRB-3 without *A. paniculata* (0.529 ± 0.067 µg/L/6.10^{5}cells). (Table 1; Figure 1) This study showed an equal insulinotropic effect of 100 ìM glibenclamide (0.844 ± 0.084 µg/L/6.10^{5}cells), 25 meq KCL (0.803 ± 0.02 µg/L/6.10^{5}cells), and 0.625 mg/mL *A. paniculata* (0.920 ± 0.07 µg/L/6.10^{5}cells).(Tabel-2) Compared to insulinotropic response of 100 ìM glibenclamide towards BRIN-BD11, 1.25 mg/mL *A. paniculata* evoked 1.48 fold (1.25 ± 0.173 µg/L/6.10^5 cells) of insulin secretion, and the greater response was 2.34 fold (1.972 ± 0.133 µg/L/6.10^{5}cells) of insulin secretion was shown at 2.5 mg/mL *A. paniculata.*(Table 1; Figure 1) These findings indicated that *A. paniculata* had a very strong insulinotropic property towards BRIN-BD11 in the media containing 16.7 mM glucose. It could be assumed that *A. paniculata* was a glucose-dependent insulinotropic agent. The greater insulinotropic response of 1.25 and 2.5 mg/mL *A. paniculata* than 25 mEq KCL, could suggest that *A. paniculata* not only affected the triggering pathway of insulin secretion as 25 mEq KCL did, but also affected the amplifying pathway of insulin secretion.[33] Static insulin secretion measurement after 60 minutes incubation of BRIN-BD11 was the sum of the first and second phases of insulin secretion, and *A. paniculata* will have a clinical value as an oral herbal anti-diabetic if it can show an amplifying effect towards the first phase of insulin secretion as shown by other oral

insulin secretagogue, such as sulfonylurea including glibenclamide. Based on this reason, the study was continued by measuring the insulin secretion of BRIN-BD11 after 20 minutes incubation.

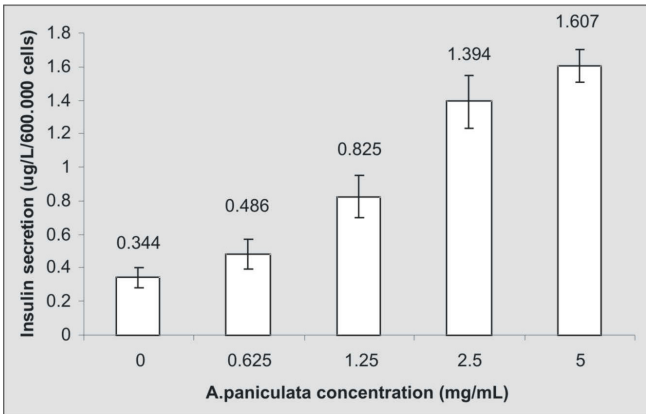

Figure 2. Static insulin measurement after 20 minutes incubation of BRIN-BD11 in media KRB-3 containing 16.7 mM glucose.(0.625–10 = *A. paniculata* concentration in mg/mL; 0 = without *A. paniculata*).

Twenty minutes incubation of BRIN-BD11 in the media KRB-3 + 0.625, 1.25, 2.5 and 5 mg/mL *A. paniculata* respectively, showed an increase of insulin secretion correspondingly with the increase of *A. paniculata* concentration. The greatest insulinotropic response, 4.7 fold of insulin secretion (1.607 ± 0.096 µg/L/6.10^5 cells) showed at 5 mg/mL *A.paniculata*, compared to insulinotropic effect of 16.7 mM glucose only ($p<0.001$), even at the lowest concentration (0.625 mg/mL), *A. paniculata* could already evoke 1.4 fold of insulin secretion (0.486 ± 0.088 µg/L/6.10^5 cells) compared to 16.7 mM glucose ($p=0.023$). (Tabel 1; Figure 2) These findings indicated that *A. paniculata* was a glucose-dependent insulinotropic agent or an insulin secretagogue in the media with high glucose concentration (16.7 mM glucose), especially in inducing the first phase of insulin secretion. This study could not determine whether *A. paniculata* itself had an insulin stimulatory effect or just amplified the insulin stimulatory effect of glucose.

Further study was continued to examine the insulin stimulatory effect of *A. paniculata* by incubating BRIN-BD11 in the media KRB-1 containing 1.11 mM glucose. In this concentration, glucose will not depolarize the BRIN-BD11 membrane, thus will not induce insulin secretion. When there was no insulin secretion in media KRB-1, it means that *A. paniculata* affected the amplifying pathway only and not the first phase or triggering pathway of insulin secretion as well as *Tinospora*

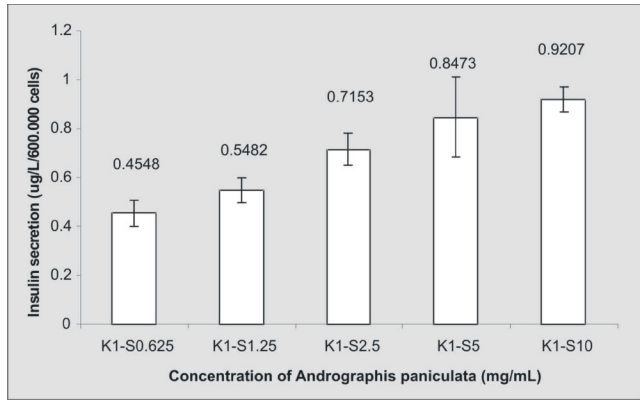

Figure 3. Static insulin measurement after 20 minutes incubation of BRIN-BD11 in media KRB-1 + various concentration of *A. paniculata* (K1= KRB-1; S0.625 – 10 = concentration of *A. paniculata* in mg/mL).

crispa.[34] The increased insulin stimulatory effect of *A. paniculata* corresponded with the increase of *A. paniculata* concentration. Twenty minutes incubation of BRIN-BD11 in the media KRB-1 containing 1.11 mM glucose, 0.625, 1.25, 2.5, 5, and 10 mg/mL *A. paniculata,* respectively, evoked 1.3 fold (0.455 ± 0.053 µg/L/6.10^{5}cells; $p = 0.019$), 1.6 fold (0.548 ± 0.051 µg/L/6.10^{5}cells; $p<0.001$), 2.1 fold (0.715 ± 0.065 µg/L/6.10^{5}cells; $p<0.001$), 2.5 fold (0.847 ± 0.162 µg/L/6.10^{5}cells; $p<0.001$), and 2.7 fold (0.921 ± 0.052 µg/L/6.10^{5}cells; $p<0.001$) of insulin secretion compared to 16.7 mM glucose only (0.344 ± 0.058 µg/L/6.10^{5}cells). (Table 3; Figure 3) These findings indicated that *A.paniculata* induced the first phase of insulin secretion and had a very strong, glucose-independent insulinotropic property as well as oral insulin secretagogue, sulfonylurea.[35, 36] From a clinical point of view, these findings indicated that *A. paniculata* pontentially induced hypoglycemia. By graphing the concentration of *A. paniculata* (mg/mL) as the X-axis, and insulin secretion (µg/L/6.10^{5}cells) as the Y-axis, a graph of the function Y is produced with the function $Y = - 0.0087 X^2 + 0.1399 X + 0.38$ ($r^2 = 0.988$). The function is very similar to the Mikaelis-Menten equation, that assumed *A. paniculata* affected the membrane receptors, one of wich was ATP-dependent potassium channels (K^+_{ATP}). (Figure 4)

The double reciprocal plot graph with (*A.paniculata* concentration)$^{-1}$ as the X-axis and (insulin secretion)$^{-1}$ as the Y-axis displays a linear function of $Y = 0.7492 X + 1.0729$ ($r^2 = 0.96$). This Figure strongly supports the assumption that the insulin stimulatory effect of *A. paniculata* occured by affecting one of the membrane receptors, mostly on K^+_{ATP}. (Figure 5)

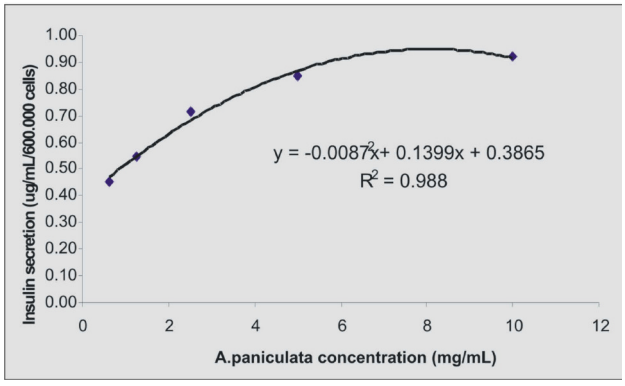

Figure 4. Graph pattern of insulin secretion of BRIN-BD11, after 20 minutes incubation in the media with various concentrations of *A. paniculata* (0.625 – 10 mg/mL)

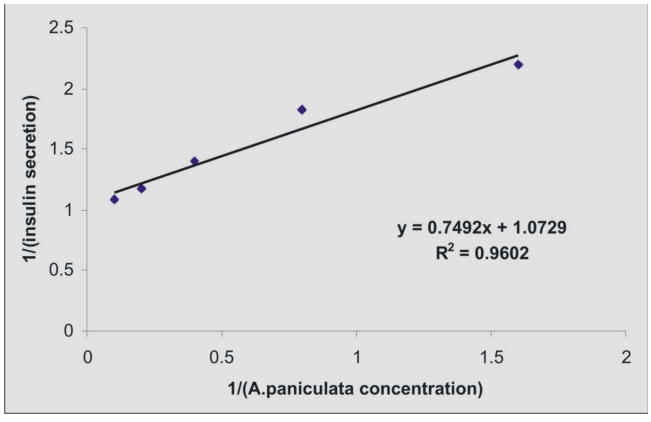

Figure 5. Double reciprocal plot of insulinotropic effect of *A. paniculata* towards BRIN-BD11 after 20 minutes incubation in the media KRB-1 containing 1.11 mM glucose

CONCLUSION

Simple water extraction or infusion of *A.paniculata* showed a very strong insulin secreting effect towards BRIN-BD11. The insulin stimulatory effect of *A.paniculata* showed as a glucose-independent and dose dependent insulin secreting agent, stimulated the first phase of insulin secretion, and mostly affected ATP-dependent potassium channels, K^+_{ATP}. As an insulin secretogogue, *A.paniculata* potentially induced hypoglycemia.

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