

# Anthocyanins-rich extract of wild Chinese blueberry protects glucolipotoxicity-induced INS832/13 $\beta$ -cell against dysfunction and death

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**Abstract** As commonly observed events in diabetic patients, glucolipotoxicity induces oxidative stress, apoptosis and functional defects in  $\beta$ -cells. Anthocyanins are well investigated as strong antioxidants and modulators for metabolic syndromes. Therefore, this study examined the protective effects of anthocyanins-rich extracts (BAE) from wild Chinese blueberry (*Vaccinium spp.*) against glucolipotoxicity in  $\beta$ -cells. Results showed that INS832/13  $\beta$ -cells subjected to glucolipotoxicity were significantly decreased ( $p < 0.05$ ) in cell survival rate, which were alleviated by BAE and metformin treatments. Both BAE and metformin reduced reactive oxidative species and improved the antioxidant defense system. Moreover, BAE were effective in reducing intracellular triglycerides (TG) level, restoring intracellular insulin content, lowering basal insulin secretion (BIS) and increasing glucose-stimulated insulin secretion which in turn resulted in an elevated insulin secretion index. However, metformin only demonstrated marginal effect on secretion dysfunction and had no effect ( $p > 0.05$ ) on BIS or TG. Additionally, TG levels reduced by BAE treatment were correlated with BIS ( $p < 0.01$ ,  $r = 0.9755$ ). This study has for the first time demonstrated that anthocyanin enriched extract of wild Chinese blueberry could effectively protect  $\beta$ -cells against glucolipotoxicity in vitro. These results implied the potential efficacy of BAE as a complementary measure for diabetes intervention.

**Keywords** Anthocyanins · Apoptosis ·  $\beta$ -cells · Glucolipotoxicity · Insulin secretion · Intracellular triglycerides

## Introduction

Type II diabetes (T2D) is a chronic metabolic disorder characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism (Birkenfeld and Shulman 2014). Progression from normal glucose tolerance to T2D is resulted from an excess glucose in blood and leads to  $\beta$ -cell dysfunctions and induction of oxidative stress, which is defined as glucotoxicity (Dubois et al. 2007; Robertson et al. 2004). Lipid metabolism disorder, increases free fatty acids (FFA) in serum and is confirmed to exert deleterious actions such as apoptosis and  $\beta$ -cell failure in islets (Kusminski et al. 2009), and this event is referred as lipotoxicity. As a result, once diabetes is established, chronic hyperglycemia and hyperlipidemia are synergistically toxic for  $\beta$ -cell function and viability. It is believed that lipotoxicity is deleterious in the context of concomitant hyperglycemia, which leads to the concept of glucolipotoxicity (Poitout and Robertson 2002). It has been reported that prolonged exposure of  $\beta$ -cells to the combination of elevated glucose and FFA causes increase in insulin secretion at low glucose concentration, failure in response to high glucose concentration, decrease in proinsulin biosynthesis, reduction of antioxidant status, elevation in TG content, exhaustion of insulin stores, and increase in cell death through apoptosis, in both in vitro and in vivo studies (Kusminski et al. 2009; Roche et al. 2000). Since pancreatic  $\beta$ -cell dysfunction and cell mass loss were reported to be important contributors to the evolution of T2D (Finegood et al. 2001), the usage of natural health products as complementary approaches to

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prevent the onset of the pathogenesis in glucolipotoxicity is of great concern.

Considerable evidence demonstrated that the consumption of a diet rich in antioxidants reduced the risk of diabetes (Rahimi et al. 2005). Anthocyanins, a group of water-soluble flavonoids, which are rich in various fruits and vegetables, belong to antioxidant polyphenols and are reported as potent inhibitors of lipid peroxidation compared to other classic antioxidants (Karakaya 2004). During the past decade, foods containing anthocyanins, especially berries, have been targeted in different studies for their potential benefits on cardiovascular health, both in vitro and in vivo (de Pascual-Teresa et al. 2010). It has also been well demonstrated that anthocyanins exhibited an array of other pharmacological properties, such as eye protection (Liu et al. 2010b), hypolipidemic effects (Yang et al. 2010), and obesity control (Tsuda et al. 2006). Recent studies have showed that a diet rich in anthocyanins may prevent the development of diabetes, reduce hyperglycemia, and protect pancreas (Sancho and Pastore 2011). Supplementation of anthocyanins from Cornelian cherries is reported to ameliorate diabetes symptoms in high-fat diet induced mice, which is associated with attenuation on  $\beta$ -cell dysfunction (Jayaprakasam et al. 2006). Purple corn which enriches cyanidin-3-glucoside is found to ameliorate hyperglycemia in mice (Tsuda et al. 2003). In addition, Chinese bayberry extract, which mainly contains cyanidin-3-glucoside, protected pancreatic  $\beta$ -cells against hydrogen peroxide-induced necrosis and apoptosis via upregulating the heme oxygenase-1 gene expression (Zhang et al. 2010). In our previous study, 17 kinds of anthocyanins were identified in the anthocyanins-rich extracts from wild Chinese blueberry (*Vaccinium spp.*) (BAE), which had significant efficacy in inhibiting oleic acid-induced triglycerides (TG) accumulation in HepG2 cells (Liu et al. 2010b, 2011). However, to our best knowledge the information about protection of anthocyanins on  $\beta$ -cells against glucolipotoxicity in vitro has never been reported. Therefore, the present study was designed to determine whether BAE could exert a direct effect against  $\beta$ -cell glucolipotoxicity caused by high levels of glucose and FFA.

## Materials and methods

### Chemicals and plant materials for test

Oleic acid (OA), palmitic acid (PA), metformin, propidium iodide (PI), RNase, RPMI-1640, 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), 2',7'-dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Gibco Life Technologies (Grand Island, NY). FFA-free bovine serum albumin (BSA) was from

Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bicinchoninic acid assay (BCA) kit was purchased from Beyotime (Haimen, China). Insulin radioimmunoassay (RIA) kit was obtained from Beijing Atom Hightech Company (Beijing, China). The TG, MDA, SOD and GSH-Px detection kits were supplied by JianCheng Company (Nanjing, China).

The wild Chinese blueberry (*Vaccinium spp.*), grown in the Greater Hinggan Mountains in northeast China, was provided by the Science and Technology Bureau of Greater Hinggan Mountains District and stored at  $-20\text{ }^{\circ}\text{C}$  before use. The extraction method of anthocyanins-rich fraction from wild Chinese blueberry was based on Liu et al. (2011). Briefly, fresh blueberry was extracted with methanol and evaporated, followed by an application on Amberlite XAD-7 column. After 1 h, the XAD was washed with 1 % aqueous formic acid solution, and then the polyphenolics were eluted with methanol. The eluent was concentrated and freeze dried to obtain the extract powder which was resolubilized and applied to a Sephadex LH20 column and eluted with 70 % methanol acidified with 10 % formic acid. The resultant eluents were freeze dried and applied to an Oasis HLB cartridge, to obtain anthocyanins-rich fractions by washing off through methanol with 10 % formic acid before freeze dried to achieve the BAE. BAE was dissolved in dimethyl sulfoxide (DMSO), with the final concentration of DMSO in cell-assays being less than 0.1 %. There are 17 anthocyanins in BAE and the total content of anthocyanins in BAE is approximately 41.0 mg/100 mg dry weight determined by pH differential method (Liu et al. 2011). Anthocyanins of malvidin glycosylated with hexose or pentose account for >46 % of total anthocyanin content. In addition, both delphinidin glycosides and petunidin glycosides account for about 20 % of the total anthocyanin content, respectively (Liu et al. 2011).

### BSA-bound fatty acids preparation

Stock solution of 1 mM FFA bound to 1 % BSA was prepared. Briefly, OA and PA (molar ratio of 2:1) were completely dissolved in 50  $\mu\text{L}$  ethanol to obtain 1 mM FFA (final ethanol concentration in treatment solution was less than 0.1 %), and 80  $\mu\text{L}$  10 M NaOH was added. Then the saponified FFA was ultrasonicated with FFA-free RPMI-1640 medium containing 1 % BSA (the mixed medium was adjust to pH 10.0) for 40 min. Finally, the pH of BSA-FFA medium was adjusted to 7.4 and filtered through a 0.22  $\mu\text{m}$  membrane filter before stored at  $-20\text{ }^{\circ}\text{C}$ .

### Cell culture

INS-1 832/13 cells (a kind gift from Christopher B. Newgard, Duke University, NC) were cultured in RPMI 1640 medium containing 11.1 mM glucose supplemented with 10 % fetal

bovine serum, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 50  $\mu$ M  $\beta$ -mercaptoethanol, 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin at 37 °C with 5 % CO<sub>2</sub>.

The cells were seeded at a density of  $2 \times 10^5$  cells/mL and incubated at 37 °C for 24 h. After the culture establishment period, the cells were washed with phosphate-buffered saline (PBS) and then incubated with FBS-free RPMI-1640 with 5.6 mM glucose at 37 °C for 12 h. After removing the pretreated medium, the cells were incubated in medium of 0.5 % BSA added with low dosage of glucose, high dosage of glucose plus 0.5 mM FFA, or high dosage of glucose plus 0.5 mM FFA with various concentrations of BAE or metformin.

#### Evaluation of insulin secretory function

Insulin secretion in response to 2.8 mM or 16.7 mM glucose after 24 h or 36 h treatment was measured by RIA kit. Cells were washed twice with HEPES balanced salt solution (HBSS, pH 7.4), and pre-incubated in HBSS buffer containing 2.8 mM glucose and 0.1 % BSA for 2 h. Then the medium was replaced with HBSS buffer containing 2.8 mM or 16.7 mM glucose with 0.1 % BSA and the cells were incubated sequentially for 2 h. Finally, the supernatant was collected and kept at –80 °C until tested. Basal insulin secretion (BIS) and glucose-stimulated insulin secretion (GSIS) were determined by insulin release at 2.8 mM and 16.7 mM glucose, respectively. Insulin secretion index (ISI) (the ratio of GSIS to BIS), represents the secretion function of pancreatic  $\beta$ -cells (Hohmeier et al. 2000).

#### Measurement on intracellular insulin content

To extract insulin, cells were washed twice with PBS, followed by incubation in 400  $\mu$ L acid alcohol solution (75 mL ethanol, 1.5 mL 12 mol/L HCl, and 23.5 mL distilled water) overnight at 4 °C. The solution was collected and centrifuged at 10,000 rpm for 5 min at 4 °C, and the supernatants was stored at –80 °C until insulin RIA assay (Hamid et al. 2002). Cells cultured side by side under the same condition were lysed for detecting total protein with BCA kit.

#### Cell viability assay

Cell viability was determined by MTT assay. After incubation in treatment solution for 48 h, the medium was replaced with 0.5 mg/mL MTT and kept for 4 h at 37 °C in the dark. Then the MTT solution was removed and 150  $\mu$ L DMSO was added into the wells. The absorbance of the reduced intracellular formazan product was read at 570 nm on a Microplate plate reader (Spectra Max M2<sup>e</sup>, Molecular Devices, USA). Cell viability was calculated by the ratio of absorbance of treatment

group to that of control group, and the viability for control group was considered as 100 %.

#### Cell apoptosis analysis

Flow cytometric analysis using PI was performed to determine cell apoptosis. Cells less intensely stained than G1 cells (sub G0/G1 cells) in flow cytometric histograms were considered as apoptotic cells. Briefly, treated INS832/13 cells of about  $1 \times 10^6$  cells were collected, and washed twice with cold PBS and fixed in ice cold 70 % ethanol overnight at 4 °C. After two more washes with cold PBS, cell pellets were re-suspended in PBS with 40  $\mu$ g/mL RNase for 30 min at 37 °C, and then incubated in PBS containing 40  $\mu$ g/mL PI in the dark for 15 min at room temperature before analysis on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, USA). DNA content was analyzed for each sample by CellQuest software and data were evaluated by Cell Quest (Becton Dickinson) and ModFit LT software.

#### Analysis of intracellular reactive oxygen species (ROS)

Cells were treated in a 96 wells black plate for 24 h at 37 °C. After washed with PBS, 25  $\mu$ M DCFH-DA was added to the cells and incubated for 30 min at 37 °C, then the cells were washed thoroughly with HBSS. The fluorescence of the cells from each well was recorded at 530 nm emission and 485 nm excitation (Zhang et al. 2011).

#### Determination of intracellular TG, malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)

The treated cells were washed twice with pre-cooled PBS after incubation in treatment solution for 36 h, followed by an addition of RIPA lysis buffer. The cell lysates were centrifuged at 10,000 rpm for 5 min at 4 °C. The TG, MDA, SOD and GSH-Px in supernatant were determined by relative detection kits (Liu et al. 2011).

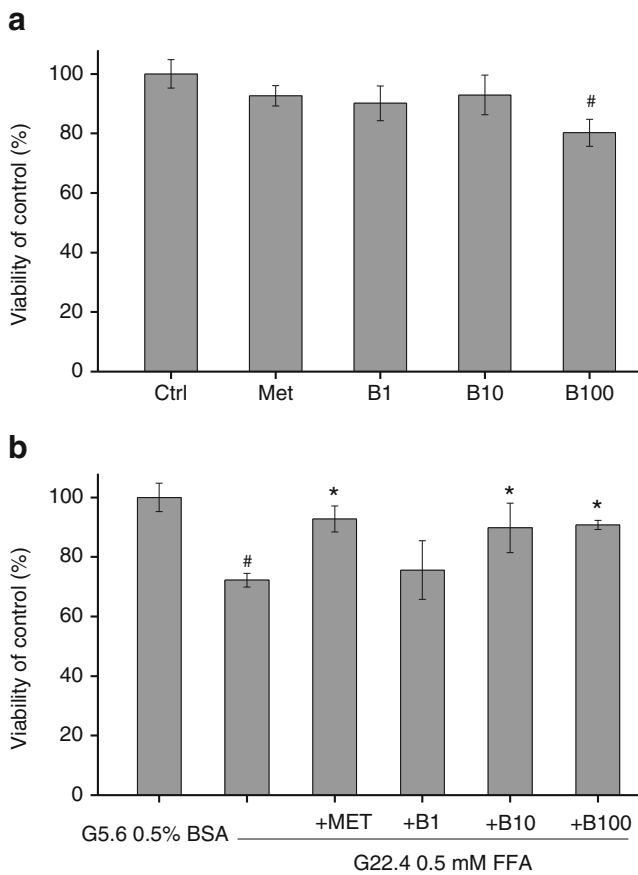
#### Statistical analysis

Data were expressed as mean values  $\pm$  standard deviation (SD) and analyzed by one-way analysis of variance followed by Tukey's post hoc test (SPSS 13.0). The significance was taken at a *P*-value of 0.05. Correlation coefficients of relative BIS of cells in different groups versus intracellular TG were determined by linear regression analysis. The significance was taken at a *P*-value of 0.01.

**Results**

**BAE protects  $\beta$ -cell viability and reduces apoptosis**

Survival of pancreatic  $\beta$ -cells is apparently of major importance for maintaining normal glucose metabolism both in type I diabetes and type II diabetes. To investigate the effect of BAE on cell mass, the viability and apoptosis were determined. As indicated in Fig. 1a, metformin, 1  $\mu\text{g}/\text{mL}$  BAE and 10  $\mu\text{g}/\text{mL}$  BAE all had no significant impacts on cell viability. However, incubation with BAE at 100  $\mu\text{g}/\text{mL}$  caused a 20 % decrease in cell viability under normal culture condition. After 48 h incubation, the elevated glucose and FFA induced a profound inhibition on cell viability by about 28 % compared to control ( $p < 0.05$ ). This decrease was altered by 2.5  $\mu\text{g}/\text{mL}$  metformin, 10  $\mu\text{g}/\text{mL}$  BAE and 100  $\mu\text{g}/\text{mL}$  BAE. Cells labeled with PI (nuclear marker for cell death) demonstrated similar results (Table 1). The apoptotic cells in model group were almost as twice as that of control. When



**Fig. 1** Cytotoxicity of metformin and various concentrations of BAE (a) and their protection on viability of cells which treated with glucolipototoxicity (b). Met: 2.5  $\mu\text{g}/\text{mL}$  metformin; B1, B10, B100: BAE at different concentrations (1, 10, 100  $\mu\text{g}/\text{mL}$ , respectively). G5.6 and G22.4 mean 5.6 mM glucose and 22.4 mM glucose, respectively. # $p < 0.05$ , means significantly different from control group. \* $p < 0.05$ , means significantly different from model group

**Table 1** Effects of metformin and BAE on intracellular insulin content, apoptosis and ROS

Group	Concentration ( $\mu\text{g}/\text{mL}$ )	Intracellular insulin content ( $\mu\text{U}/\mu\text{g pro}$ )	Apoptosis (%)	ROS (%)
Control	–	95.7 $\pm$ 4.9	3.1 $\pm$ 0.2	62.9 $\pm$ 5.2
Model	–	60.3 $\pm$ 1.5 <sup>#</sup>	6.9 $\pm$ 0.4 <sup>#</sup>	100.0 $\pm$ 7.0 <sup>#</sup>
Metformin	2.5	61.6 $\pm$ 0.2	4.4 $\pm$ 0.1*	79.1 $\pm$ 8.9*
BAE	1	69.8 $\pm$ 4.0*	5.5 $\pm$ 0.5	88.1 $\pm$ 9.8*
	10	89.4 $\pm$ 5.9*	4.8 $\pm$ 0.1*	81.0 $\pm$ 9.9*
	100	85.0 $\pm$ 6.6*	4.5 $\pm$ 0.7*	62.5 $\pm$ 8.7*

ROS was expressed as percentage of model group

# $p < 0.05$ , means significantly different from control group

\* $p < 0.05$ , means significantly different from model group

treated with metformin, 10  $\mu\text{g}/\text{mL}$  BAE or 100  $\mu\text{g}/\text{mL}$  BAE, the percentage of apoptosis was lowered significantly ( $p < 0.05$ ).

**BAE scavenges ROS and restores the antioxidant defense systems**

As expected, high levels of glucose and FFA induced more ROS production in the present study. Both metformin and BAE decreased ROS production. However, BAE decreased ROS production dose-dependently (Table 1). The presence of 100  $\mu\text{g}/\text{mL}$  BAE strongly scavenged intracellular ROS to 69 % that of the model group and reached the level to that of the control (Table 2). Intracellular activities of antioxidant enzymes such as SOD and GSH-Px, were significantly inhibited along with an elevation in MDA content after prolonged exposure to high levels of glucose and FFA ( $p < 0.05$ ). Metformin and BAE treatments all altered these abnormalities, whereas BAE

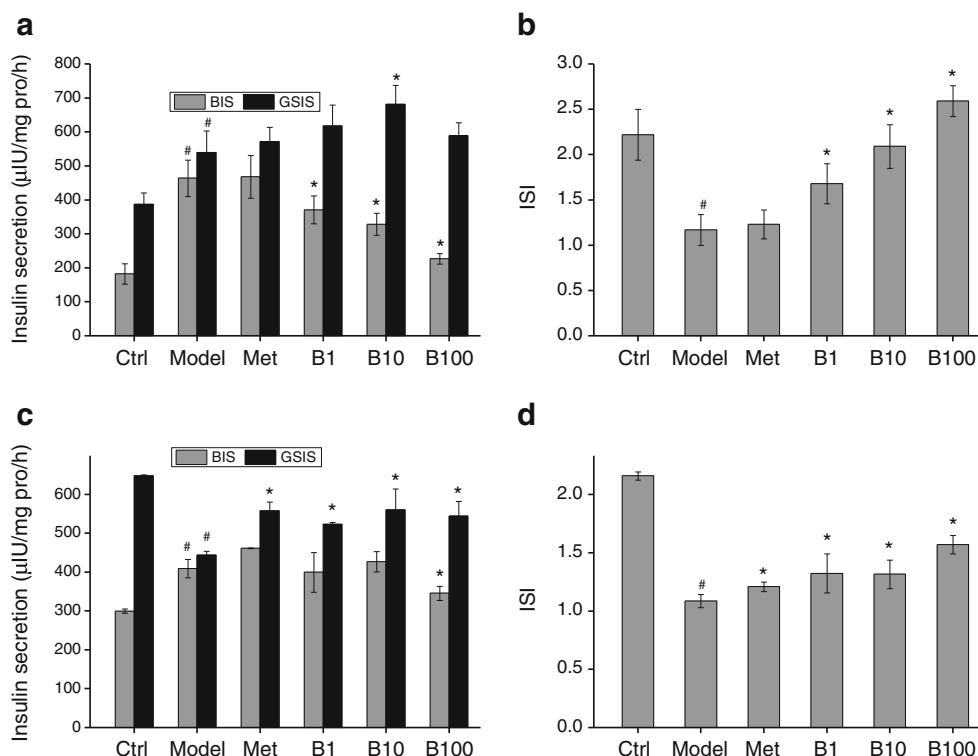
**Table 2** Effects of metformin and BAE on SOD, GSH-Px activity and MDA content

Group	Concentration ( $\mu\text{g}/\text{mL}$ )	SOD (U/g pro)	GSH-Px (U/mg pro)	MDA ( $10^{-2}$ nmol/g pro)
Control	–	16.7 $\pm$ 0.3	69.1 $\pm$ 2.5	29.1 $\pm$ 1.5
Model	–	15.3 $\pm$ 1.0 <sup>#</sup>	43.7 $\pm$ 5.8 <sup>#</sup>	38.7 $\pm$ 1.5 <sup>#</sup>
Metformin	2.5	16.6 $\pm$ 0.8*	33.3 $\pm$ 2.4*	33.3 $\pm$ 0.7*
BAE	1	16.2 $\pm$ 0.5*	62.1 $\pm$ 2.0*	29.8 $\pm$ 3.2*
	10	19.9 $\pm$ 1.0*	60.2 $\pm$ 7.5*	26.7 $\pm$ 1.7*
	100	15.6 $\pm$ 2.3	57.3 $\pm$ 2.3*	24.7 $\pm$ 0.4*

# $p < 0.05$ , means significantly different from control group

\* $p < 0.05$ , means significantly different from model group

**Fig. 2** Effects of metformin and BAE on high levels of glucose and FFA induced  $\beta$ -cell dysfunction. INS832/13 cells were treated for 24 h (a) and 36 h (c), the relative ISI were presented in b and d. Ctrl: 5.6 mM glucose and 0.5 % BSA; Model: 0.5 mM FFA and 22.4 mM glucose; Met: model cells treated with 2.5  $\mu$ g/mL metformin; B1, B10, B100: model cells treated with BAE at different concentrations (1, 10, 100  $\mu$ g/mL, respectively); ISI insulin secretion index. # $p$ <0.05, means significantly different from control group. \* $p$ <0.05, means significantly different from model group



showed better improvement on GSH-Px activity than that of metformin.

#### Protective effect of BAE on intracellular content and insulin secretion function

Based on intracellular insulin measurements, it was found that the insulin content in model group remarkably dropped to 63 % of control group after 36 h treatment (Table 1). In comparison, the intracellular insulin contents of the model cells treated with different doses of BAE were significantly recovered ( $p$ <0.05), with the insulin content reached to the maximum level (93 % of control) at 10  $\mu$ g/mL of BAE. However, metformin had no obvious effect on insulin levels.

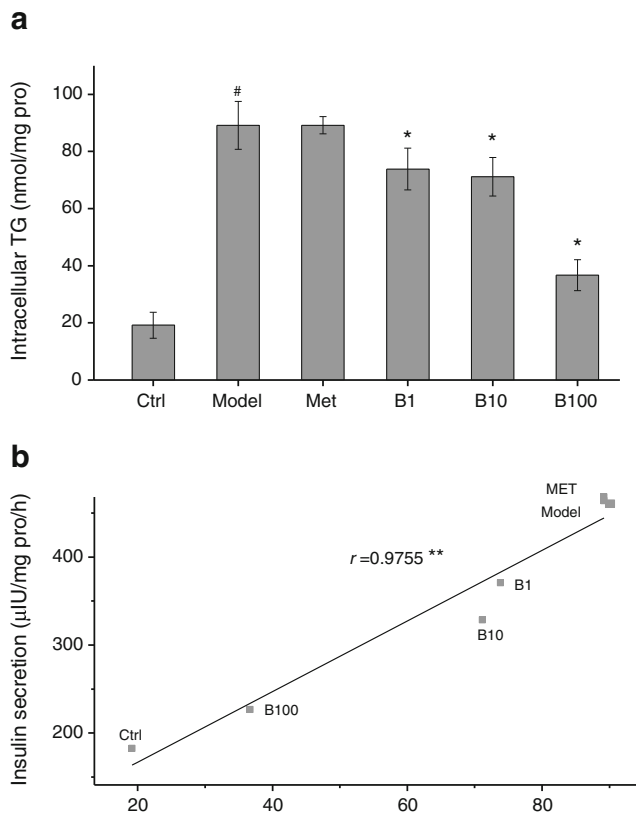
Twenty-four and 36 h exposure to 22.4 mM glucose and 0.5 mM FFA led to significant increases in BIS compared to control (Fig. 2a and c;  $p$ <0.05), and 36 h exposure also induced a dramatic decrease in GSIS (Fig. 2c;  $p$ <0.05). In the present study, after incubation with different concentrations of BAE for 24 h, the BIS of cells were dose-dependently inhibited and 100  $\mu$ g/mL BAE markedly reduced BIS to 50 % of that of model ( $p$ <0.05), while at 36 h BAE exhibited significant stimulating effect in GSIS (Fig. 2a and c;  $p$ <0.05). However, metformin failed to lower the BIS during the experiment. At both 24 and 36 h (Fig. 2b and d), glucolipototoxicity reduced ISI to almost half that of the control cells, while BAE at all concentrations could enhance ISI.

BAE reduced intracellular TG content and this effect was correlated with BIS

The effect of different BAE concentrations on TG deposition was shown in Fig. 3a. Intracellular TG of model group was about five times that of the control. BAE at 1  $\mu$ g/mL could effectively reduce intracellular TG accumulation to approximately 83 % in comparison with model. When treated with 100  $\mu$ g/mL BAE, intracellular TG was effectively inhibited by about 40 % ( $p$ <0.05; compared to model group), whereas no effect by metformin was observed. After analysis, TG content and BIS value was found to be significantly correlated ( $r$ =0.9755,  $p$ <0.01).

#### Discussion

In the present study, BAE treatment on regular cells induced a mild cytotoxicity, which might be due to the anti-cancer ability of anthocyanins because INS832/13 cell line was established from INS-1 cancer cell line (Liu et al. 2010a; Hohmeier and Newgard 2004). Although 100  $\mu$ g/mL BAE caused 20 % cell death in regular cell culture medium, we still used this concentration in regarding its strong effectiveness on cell protection in the medium that could cause glucolipototoxicity. It is demonstrated that BAE significantly reduced the apoptosis induced by glucolipototoxicity in a dose-dependent manner, implying that BAE increased cell survival



**Fig. 3** Effects of metformin and BAE on high levels of glucose and FFA induced  $\beta$ -cell TG accumulation (**a**) and the correlations between TG values and BIS values (**b**). INS832/13 cells were treated for 24 h. Ctrl: 5.6 mM glucose and 0.5 % BSA; Model: 0.5 mM FFA and 22.4 mM glucose; Met: model cells treated with 2.5  $\mu$ g/mL metformin; B1, B10, B100: model cells treated with BAE at different concentrations (1, 10, 100  $\mu$ g/mL, respectively). <sup>#</sup> $p < 0.05$ , means significantly different from control group. <sup>\*</sup> $p < 0.05$ , means significantly different from model group. <sup>\*\*</sup> $p < 0.01$  indicates significant linear correlation

is partially associated with its ability against apoptosis. In vitro, prolonged exposure to either 2 mM FFA or 16.7 mM glucose induced apoptosis in isolated islets by increasing Bax (proapoptotic gene) expression, reducing Bcl-2 (antiapoptotic gene) expression and slightly increasing caspase-3 expression (Piro et al. 2002). It was reported that anthocyanins of black soybean seed coats, mainly cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside, could regulate caspase-3, Bax and Bcl-2 proteins to protect pancreatic tissue from streptozotocin-induced apoptosis in diabetic rats (Nizamutdinova et al. 2009), which further indicated that anthocyanins of BAE might protect  $\beta$ -cell against glucolipotoxicity by the same mechanism.

In the development of pathologic states of diabetes, over production of ROS and disturbed capacity of antioxidant defense in diabetic subjects have been well documented (Kawahito et al. 2009). Increased metabolism of glucose and FFA through mitochondrial oxidation results in an elevated mitochondrial membrane potential and superoxide production (Lowell and Shulman 2005), and the increased ROS could

react with lipids and eventually leads to elevated lipid peroxidation. Our results showed that prolonged exposure to high levels of glucose and FFA could successfully induce damage towards the antioxidant defense system, which was similar to the reports in vitro (Nizamutdinova et al. 2009). Theoretically, the oxidative stress caused by elevated FFA or glucose levels might be diminished by antioxidants such as anthocyanins. As expected, treatment of BAE reduced ROS production and altered the antioxidant system and inhibited the lipid peroxidation products which might be due to their promising ability of scavenging free radicals (Wang et al. 1999).

Altered insulin secretion function when treated with high levels of glucose and FFA for 24 h, implied that these nutrient factors stimulated the insulin secretion through both BIS and GSIS as speculated, but the significant decrease in relative ISI (an index for insulin secretion) indicated impairments in cell functions. After prolonged incubation of 36 h,  $\beta$ -cells were adapted or could compensate insulin secretion under low glucose concentration. Therefore, increased insulin secretion in turn resulted in a decrease in the intracellular insulin stores, which could not be offset by commensurate nutrients induction of proinsulin biosynthesis (Bollheimer et al. 1998) and might further affect the insulin secretion towards high glucose concentration. As expected, at 36 h, cells lost its sensitiveness to high glucose exposure, indicating the appearance of defective GSIS. A similar result was reported that BIS was enhanced while GSIS was diminished in isolated Sprague–Dawley rat islets when exposed to palmitate, oleate, or octanoate for periods of up to 48 h (Zhou and Grill 1994). Evidence in the present work showed that BAE at all concentrations could enhance the ISI, suggesting its potency on improvements of the insulin secretion function of cells. Earlier reports indicated that anthocyanins such as cyanidin-3-glucoside, pelargonidin-3-galactoside and delphinidin-3-glucose were effective insulin secretagogues for INS832/13 cells in the presence of 4 and 10 mmol/L glucose concentrations (Jayaprakasam et al. 2005). It was reported that C57BL/6 mice fed with high-fat diet developed elevated blood glucose and this was prevented by anthocyanins from Cornelian cherry (*Cornus mas*) due to their ability of stimulating insulin secretion to alleviate insulin resistance (Jayaprakasam et al. 2006). Similar anthocyanins were presented in BAE, therefore it might have the potential to stimulate GSIS in  $\beta$ -cells under glucolipotoxicity.

High plasma levels of FFA and enhanced activity of lipogenesis cause TG deposition in cells (Poitout and Robertson 2008). Excess TG deposition in  $\beta$ -cells affects energy metabolism and impairs insulin secretion (Hirose et al. 1996). We found that intervention by BAE significantly inhibited the TG accumulation in the cells. Metformin could slightly increase ISI and significantly ( $p < 0.05$ ) reduce ROS production, however, it did not inhibit TG accumulation, which was in accordance with a previous report (Lupi et al. 2002). However, the

possible mechanism for BAE and metformin to act differently is still unknown. Overall, BAE exerted better comprehensive effects over the anti-diabetic drug, metformin.

Interestingly, in the present study, the TG content and BIS value was found to possess notable correlations ( $r=0.9755$ ,  $p<0.01$ ). A few reports claimed that overexpression of hexokinases, but not glucokinase, significantly enhances BIS of islets (Becker et al. 1994; O'Doherty et al. 1996). High carbohydrate diet causes islet dysfunction during the neonatal period in rats, and it appears that the marked increased hexokinase activity coupled with a significant increase in hexokinase protein content of islets plays a significant role in sustaining basal hyperinsulinemia (Aalinkeel et al. 1999). Hexokinase converts glucose to glucose-6-phosphate and initiates glucose metabolism (Osawa et al. 1996), thus elevated glucose may upregulate the activity of this enzyme, thereby increases glucose phosphorylation and glycerol synthesis to promote TG synthesis with FFA (Becker et al. 1994). BIS involves in glucose sensing, insulin producing and secreting, and its elevation may contribute to the formation of hyperinsulinemia, eventually aggravates insulin resistance and promotes onset of diabetes (Zhang et al. 2005). Thus it was of interest to investigate if nutrients could inhibit TG accumulation and whether they may be also effective to reduce BIS. The mechanisms underlying the relationship of BIS regulation and TG content needed further investigation.

## Conclusion

Glucolipotoxicity of INS832/13  $\beta$ -cells could be induced by high levels of glucose and FFA and intervened by BAE or antidiabetic drug metformin. BAE significantly increased cell viability by decreased apoptosis, reduced cell secretion dysfunction, scavenged intracellular ROS, elevated antioxidant status and prevention in intracellular TG accumulation. In comparison, metformin manifested positive effects on cell survival and antioxidant defense system, only a marginal effect on the cell secretion dysfunction, but no apparent effect on BIS or TG. Moreover, the TG content in cells were found to be correlated with BIS value ( $p<0.01$ ,  $r=0.9755$ ). Overall, BAE showed more comprehensive and beneficial effects on protecting  $\beta$ -cells against glucolipotoxicity over metformin.

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