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Muscadine or amla extracts standardized to ellagic acid content ameliorate glucolipotoxicity associated β-cell dysfunction via inhibition of IL-1β and improved insulin secretion

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Abstract

Glucolipotocixity induces IL-1 β secretion which impairs pancreatic β-cell insulin secretion. Ellagic acid and urolithin A have strong anti-inflammatory effect on cells. Muscadine and amla are very good sources of ellagic acid. The present study examined the effect of ellagic acid, ellagic acid-rich muscadine or amla extract, or urolothin A on inflammation in β cells under glucolipotoxic conditions. Rat NIT-1 β cells were incubated in glucolipotoxic conditions (33.3 mM glucose, 250 μM palmitic acid or 33.3 mM glucose + 250 μM palmitic acid with or without ellagic acid, ellagic acid-rich muscadine or amla extracts standardized to its ellagic acid content, or urolithin A). Inflammatory status was evidenced by ELISA analysis of insulin and IL-1β secretion. Ellagic acid-rich muscadine or amla extracts dose-dependently stimulated insulin secretion and down-regulated IL-1β better than pure ellagic acid, or urolithin A. Urolithin A did not statistically stimulate insulin secretion and did not inhibit IL-1β.

Keywords: Muscadine, Amla, Ellagic acid, NIT-1 pancreatic β-cells; glucose, Palmitic acid, Glucolipotoxicity, IL-1β, Inflammation, Inflammasome, Insulin secretion

Introduction

Glycolipotoxicity is the synergistic cytotoxic effect of hyperglycemia and hyperlipidemia to pancreatic β-cells (Donath et al. 2009; El-Assaad et al. 2003; Lundh et al. 2013). Hyperglycemia induces oxidative stress and is cytotoxic to pancreatic β-cells. Hyperlipidemia induced by saturated fatty acids such as palmitate, linoleate or stearate is cytotoxic to human pancreatic β-cells (Carpentier et al. 1999; Kashyap et al. 2003). Glycolipotocixity stimulates the generation of pro-inflammatory IL-1β (Donath et al. 2009). High levels of IL-1β cause faulty insulin secretion (Böni-Schnetzler et al. 2008) and ultimately β-cell impaired function (Kathrin Maedler et al. 2002). Molecules including dietary bioactive molecules that inhibit glycolipotoxicity-induced β-cell impairment are needed. Muscadine (*Vitis rotundifolia*) and amla (*Emblica officinalis*) also known as Indian gooseberry are good sources of ellagic acid whose anti-oxidative, hypoglycemic, and insulin stimulating effects have been demonstrated in rat models of type 2 diabetes (Ríos et al. 2018; Tomás-Barberán et al. 2009).

Muscadine is a good source of ellagic acid and quercetin (Talcott and Lee 2002; Yi et al. 2005). The anti-diabetic activity of muscadine has been demonstrated in vivo and muscadine inhibits the formation of advanced glycation

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end products (Farrar 2006; Wang et al. 2011) and enzymes related to carbohydrate metabolism (You et al. 2012). However, the mechanism of anti-diabetes activity of muscadine needs to be identified.

Amla (Indian gooseberry) is a good source of ellagic acid and quercetin and has anti-inflammatory, antioxidative properties, and anti-diabetic effects in rats or humans (Yadav et al. 2017) or human subjects .

The objective of the present study was to investigate the effect of ellagic acid, muscadine or amla extracts standardized to their ellagic acid content or ellagic acid metabolite urolithin A on glucose-, palmitic acid- or glucose + palmitic acid-induced IL-1β and insulin secretion by NIT-1 pancreatic β-cells.

Materials and methods
Materials
Glucose (Glu), palmitic acid (PA), ellagic acid (EA), and bovine serum albumin (BSA) were obtained from Sligma-Aldrich (St. Louis, MO). Urolithin A (UR) was a gift from Dr. Tomas Barberan, F.A., (CEBAS, Spain). F12k medium was obtained from ATCC (Manassas, VA) and fetal bovine serum (FBS) was purchased from AT-LANTA® Biologicals (Lawrencevillie, GA). ELISA kits were purchased from Crystalchem (Downers Grove, IL). All other reagents were commercial grade and purchased form Fisher Scientific (Fair Lawn, NJ). Ison variety of muscadine (MS) pomace was obtained from Feliciana Cellars (Jackson, LA) and frozen fruits of amla (AM) were purchased from Deep Foods Inc. (Union, NJ).

Muscadine or Amla extract preparation
MS pomaces or AM fruits were blended, freeze dried and stored at − 20 °C until use. Five grams of freeze-dried MS or AM was taken and dissolved in 100 ml of 80% methanol + 20% 6 N HCl. Acid hydrolysis was carried out in a water bath (Labline orbit microprocessor shaker bath) at 60 °C and 200 rpm for 2 h for the conversion of flavonoid glycosides to aglycones. Samples were then sonicated (Branson 2510, Danbury, CT) for 10 min to maximize the extraction (Pastrana-Bonilla et al. 2003). The extracts were vacuum filtered through a Whatman No.1 filter paper using a Buchner funnel. The solvents were removed in a rotary evaporator (Buchi Rotavapor, New Castle, DE) and the extracts were neutralized with 1 N NaOH. The resultant extracts were freeze dried (Genesis 35 XL lyophilizer, VirTis Co., NY) and stored at − 20 °C until use. The dried extract was extracted with ethyl acetate solvents, and dried under a stream of nitrogen gas.

Determination of ellagic acid content in muscadine or amla extracts
Agilent 1100 series HPLC (Agilent, Santa Carla, CA) equipped with an Agilent Zorbax SB-C18 column (5 μm, 4.6 × 250 mm) connected to a G1315B Diode Array UV Detector was used to determine the ellagic acid content in the fruit extracts. Phenolic acids in MS or AM extracts were separated and analyzed using the method of Pastrana et al. with modifications (Pastrana-Bonilla et al. 2003). One mg of freeze dried MS or AM extract was dissolved in 1 ml of 80% methanol + 20% 6 N HCl and filtered through a 0.22 μm nylon syringe filter before injecting into HPLC. Three mobile phases were used namely, solvent A, methanol/acetic acid/water (10,2:88, v/v/v); solvent B, acetonitrile; and, solvent C, water. All the solvents were filtered through 0.2 μm filter paper and sonicated for 10 min prior to use.

Gradient elution was performed as follows: at 0 min, 100% solvent A; at 5 min, 90% solvent A and 10% solvent B; at 25 min, 30% solvent A and 70% solvent B; at 30 min, 100% solvent C; at 35 min, 100% solvent C; at 36 min, 100% solvent A and 4 min postrun with 100% solvent A. Twenty μL of sample was injected, the column temperature was maintained at 40 °C and the flow rate was 1 mL/min. The detection was done at 280 nm using a diode array detector (DAD) (Sandhu and Gu 2010). The samples were run in triplicates and peaks and concentration of ellagic acid in fruit extracts were confirmed by matching with the retention times obtained by running the calibration curve of ellagic acid standard.

Cell culture
NIT-1, a pancreatic β-cell line developed from non-obese diabetic (NOD) mice, *Mus musculus* was obtained from ATCC (Manassas, VA). The cells were cultured and maintained in F12k medium containing 10% FBS, 2% sodium bicarbonate and 1% glutamate.

Conjugation of palmitic acid with bovine serum albumin (PA-BSA)
The conjugate of PA with BSA was prepared as described (McIntosh 2012). Thirty percent BSA was prepared in Dulbecco's Phosphate Buffered Saline (DPBS) that contained 2.5% HEPES. The pH of the solution was adjusted to 7.2 with 1 M KOH and then sterile-filtered through a 0.22 μm syringe filter. A stock solution of 12.5 mM PA was prepared before conjugation with BSA. Ten mg of cell culture grade PA was aseptically weighed and dissolved in hexane at 9% of final volume of stock. The mixture was vortexed for few seconds and then dried under nitrogen to obtain a white, chalky powder. The salt thus obtained was immediately redissolved in warm sterile water at 9% of final volume of stock and combined with 30% BSA at 91% of final stock volume. The resultant conjugate of PA was flushed with argon, aliquoted into sterile vials and stored at -20 °C until use.

MTS cell viability assay
NIT-1 cells were seeded at 10,000 cells per well in 96-well plate and incubated for 24 h before treatment. A

stock solution of EA was prepared in methanol and stock solutions of MS and AM were prepared in DMSO. Stock solutions were diluted with medium to obtain concentrations of 100 μM or 1 μM before cell treatment. Glu stock solution was prepared in the medium and sterile-filtered through a 0.22 μm nylon syringe filter. Cells were treated with 33.3 mM Glu, 250 μM PA, or a combination of 33.3 mM Glu and 250 μM PA and incubated for 24 h at 37 °C with 5% CO_2. Thereafter 0.01 μM, 0.05 μM, 1 μM or 10 μM of EA, or MS or AM extracts containing 0.01 μM, 0.05 μM, 1 μM or 10 μM equivalent of EA were added to the cells followed by incubation at 37 °C, 5% CO_2, for 72 h. Similarly, 0.01, 0.05, 1 or 10 μM of UR was added to the cells followed by incubation for 72 h. Cell viability was determined after a total incubation of 96 h using the MTS assay with CellTiter 96 A_{queous} One solution (Promega, Madison, WI) according to the manufacturer's protocol. Absorbance values of the MTS assays were read on a Bio-Rad Model 680 micro plate reader (Hercules, CA). All the treatments were performed in triplicates and the results are presented as percentage of control.

Interaction between inducers and inhibitors of inflammation in NIT-1 cells

To study the effect of the inducer (Glu, PA or Glu + PA) and inhibitor (EA, MS, AM or UR) on inflammatory markers, 3×10^5 NIT-1 cells were seeded in 6-well plates and incubated for 24 h in a humidified atmosphere at 37 °C with 5% CO_2. The cells were treated with 33.3 mM Glu, 250 μM PA or combination of 33.3 mM Glu and 250 μM PA for 24 h. Then, 0.01 μM, 0.05 μM, 1 μM or 10 μM of EA, or MS or AM containing 0.01 μM, 0.05 μM, 1 μM or 10 μM equivalent of EA were added to the wells followed by incubation for 72 h. Similarly, 0.01, 0.05, 1 or 10 μM of UR was added to the wells followed by incubation for 72 h. The supernatants were removed and stored at -80 °C until further analysis. All the treatments were performed in duplicates.

Analysis of IL-1β and insulin secretion

IL-1β levels in the supernatants of control or treated cells were analyzed by sandwich ELISA using commercially available kits from Peprotech (Rock Hill, NJ) according to the manufacturer's instructions. Insulin secretion levels in the supernatants of control or treated cells were analyzed using the commercially available ultra-sensitive mouse insulin ELISA kit from Crystalchem (Downers Grove, IL). All assays were performed according to the manufacturer's protocol.

Statistical analysis

Data were expressed as means ± standard deviation. Statistical analysis was performed using the Statistical Analysis Software (SAS) (version 9.3). Differences between control and treatments were determined by analysis of variance (ANOVA) and followed by Tukey analysis. A *P*-value of < 0.05 was considered statistically significant.

Results

Ellagic acid content in muscadine or amla extracts

EA content in MS and AM is presented in Fig. 1a. Ellagic acid, myricetin, quercetin and kaempferol were major phenolics in MS skins (Pastrana-Bonilla et al. 2003). Gallic acid and ellagic acid were major phenolics in AM (Mirunalini and Krishnaveni 2010; Pozharitskaya et al. 2007). Quercetin is a minor phenolic in AM (Mirunalini and Krishnaveni 2010). The range of EA concentration in MS was 36–91 mg/100 g on F.W. basis (Törrönen 2009). EA content in Ison variety of MS was 8.7 mg/100 g F. W in whole fruit and 22 mg/100 g of F. W in skins alone (Pastrana-Bonilla et al. 2003). HPLC analysis of commercially available extracts of AM showed that EA content was between 1.29 and 2.42 mg/g (Poltanov et al. 2009). Thin layer chromatographic (TLC) analysis of commercially available water extracts of AM showed that the ellagic acid content in AM was 6.45 g/100 g F.W. (Pozharitskaya et al. 2007). The variation in the amount of ellagic acid among different studies may be due to the differences in cultivars, growing conditions and harvest time (Lee and Talcott 2004).

Effect of EA, MS, AM or UR on the viability of NIT-1 cells treated with Glu, PA or Glu + PA

The results of cell viability are presented in Fig. 2.. The concentrations tested in the study included the range of plasma levels of EA (0.1 μM - 0.7 μM) reported in the literature (Hamad et al. 2009; Seeram et al. 2004). EA, MS or AM extracts, or UR were not cytitoxic to NIT-1 cells incubated with Glu (Fig. 2). The observed dose-dependent decrease in cell viability in the present study is similar to the reported increase in cell death from 3.8% at higher dilution of muscadine skin extract of 1:400 to 8.7% at a lower dilution of 1:100 (Greenspan et al. 2005). A potential explanation is the difference in response from the bioactive compounds at different doses, these bioactives can be stimulatory at low concentration and toxic at high concentration (Calabrese et al. 2007), a phenomenon known as "hormetic effect" that is characteristic of several dietary antioxidants (Mattson and Cheng 2006). The viability of cells exposed to PA or Glu + PA and treated with EA, MS or AM extracts, or UR showed that there was no significant difference in cell viability compared to control cells. The concentrations (0.1-10 μM) tested either showed proliferative effect or no effect on NIT-1 cell viability. The dose-dependent decrease in cell viability was similar to the response observed with Glu and EA, MS or AM extracts, or UR -treated NIT-1 cells (Fig. 2). The bioavailability of UR differed among studies, one study reported the
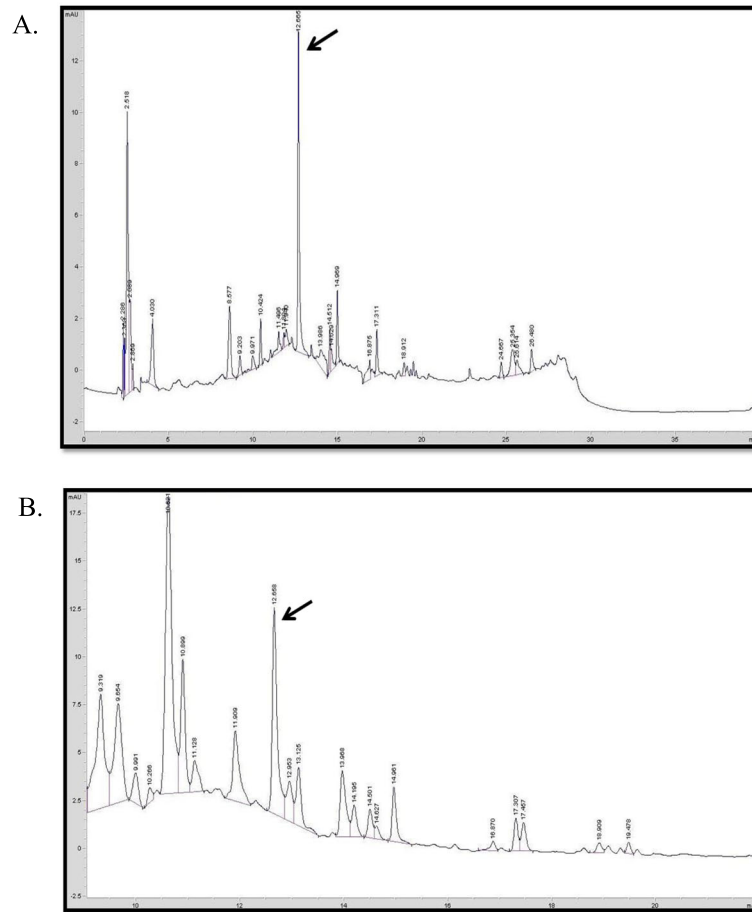
Fig. 1 a HPLC profile of muscadine fruit extract. EA eluted at 12.6 min. **b** HPLC profile of amla fruit extract. EA eluted at 12.6 min.

maximum attainable level as 5 μM (Cerda et al. 2004), whereas another study reported a range of 14–25 μM (Cerdá et al. 2004).

Effect of EA, MS, AM or UR on Glu-induced IL-1β secretion
Exposure to 33.3 mM Glu significantly increased ($P < 0.05$) the levels of IL-1β secretion by $38.29 \pm 1.85\%$ compared to control (Fig. 3a). Similar results on the increase of IL-1β levels in cells exposed to glucose concentrations higher than 11 mM have been reported (Kathrin Maedler et al. 2002). IL-1β is produced in response to high concentrations of glucose or free fatty acids (Donath et al. 2009). Treatment with EA, MS or AM showed significant ($P < 0.05$) dose-dependent inhibition of IL-1β.

EA at 0.01 μM- 10 μM significantly ($P < 0.05$) inhibited IL-1β secretion. At nanomolar concentrations of EA, IL-1β levels were equal to that of control and the maximum inhibition was observed at 1 μM of EA when IL-1β was reduced by $80 \pm 3.4\%$ of control. The mechanism by which EA suppressed IL-1β may be associated with the inhibition of oxidative stress and subsequent attenuation of inflammatory cytokines. MS or AM containing EA

equivalent dose-dependently inhibited IL-1β similar to pure EA. IL-1β was significantly ($P < 0.05$) inhibited in cells treated with MS, whereas AM showed significant inhibition only at 1 μM and 10 μM, respectively. MS or AM containing 10 μM equivalent EA inhibited IL-1β by $89.6 \pm 1.6\%$ or $68.3 \pm 1.7\%$, respectively compared to the control. MS skin extracts containing ellagic acid equivalents ranging between 0.56 μM and 2.23 μM dose-dependently inhibited IL-1β in LPS-treated blood mononuclear cells (Greenspan et al. 2005).

UR had no inhibitory effect on IL-1β induced by Glu (Fig. 3a). The low antioxidant potential of UR compared to its parent compound EA may have been associated with the inability of UR to inhibit IL-1β (Landete 2011). The high reactivity of EA compared to UR may be explained on the basis of the presence of 4 hydroxyl groups in the EA molecule compared to 2 hydroxyl groups in the UR molecule. It can also be suggested that urolithin B which has only 1 hydroxyl group in its structure should be even weaker than urolithin A against IL-1β. The presence of reduced hydroxyl groups on the urolithin A or B molecule may explain the low efficacy
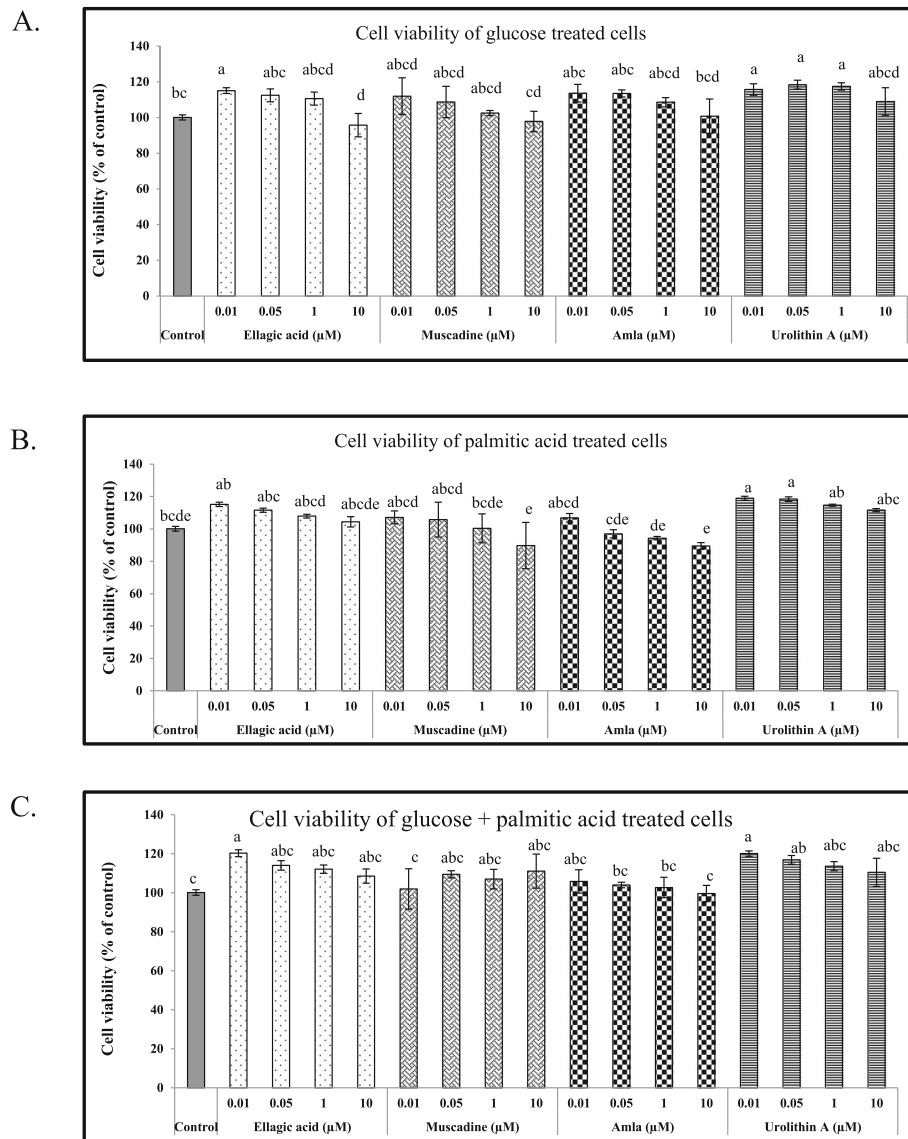
Fig. 2 a Effects of glucose (Glu), ellagic acid (EA), muscadine (MS) amla (AM), or urolithin A (UR) on the viability of NIT-1 cells. **b** Effects of palmitic acid (PA), ellagic acid (EA), muscadine (MS), amla (AM),, or urolithin A (UR) on the viability of NIT-1 cells. **c** Effects of glucose (Glu) + palmitic acid (PA), ellagic acid (EA), muscadine (MS), amla (AM), or urolithin A (UR) on the viability of NIT-1 cells. The values are expressed as percentage of untreated control. Results are presented as mean ± S.D., (*n* = 3)

of these compounds compared to their parent compound EA on cells such as NIT-1 cells.

Effect of EA, MS, AM or UR on PA-induced IL-1β secretion

PA treatment increased IL-1β secretion by 6.84 ± 0.88% compared to control. However, the increase was not statistically significant (Fig. 3b). PA-induced IL-1β secretion was less than Glu-induced IL-1β (38.29 ± 1.85%). BSA treatment did not increase IL-1β level compared to control thereby confirming that PA induced IL-1β secretion.

EA at 1 μM to 10 μM dose-dependently and significantly (*P* < 0.05) inhibited IL-1β (Fig. 3b). EA at 10 μM

reduced IL-1β secretion to 26.96 ± 4.95% of the control. All of the extracts of MS or AM containing various concentrations of ellagic acid dose-dependently inhibited IL-1β. MS or AM containing 10 μM EA equivalent inhibited IL-1β by 95 and 99% of control, respectively. The higher inhibition of IL-1β by MS or AM containing EA equivalent compared to pure EA may be due to the synergistic effects of unidentified compounds other than EA in MS or AM. AM contains anti-oxidants that inhibit IL-1β (Rao et al. 2005).

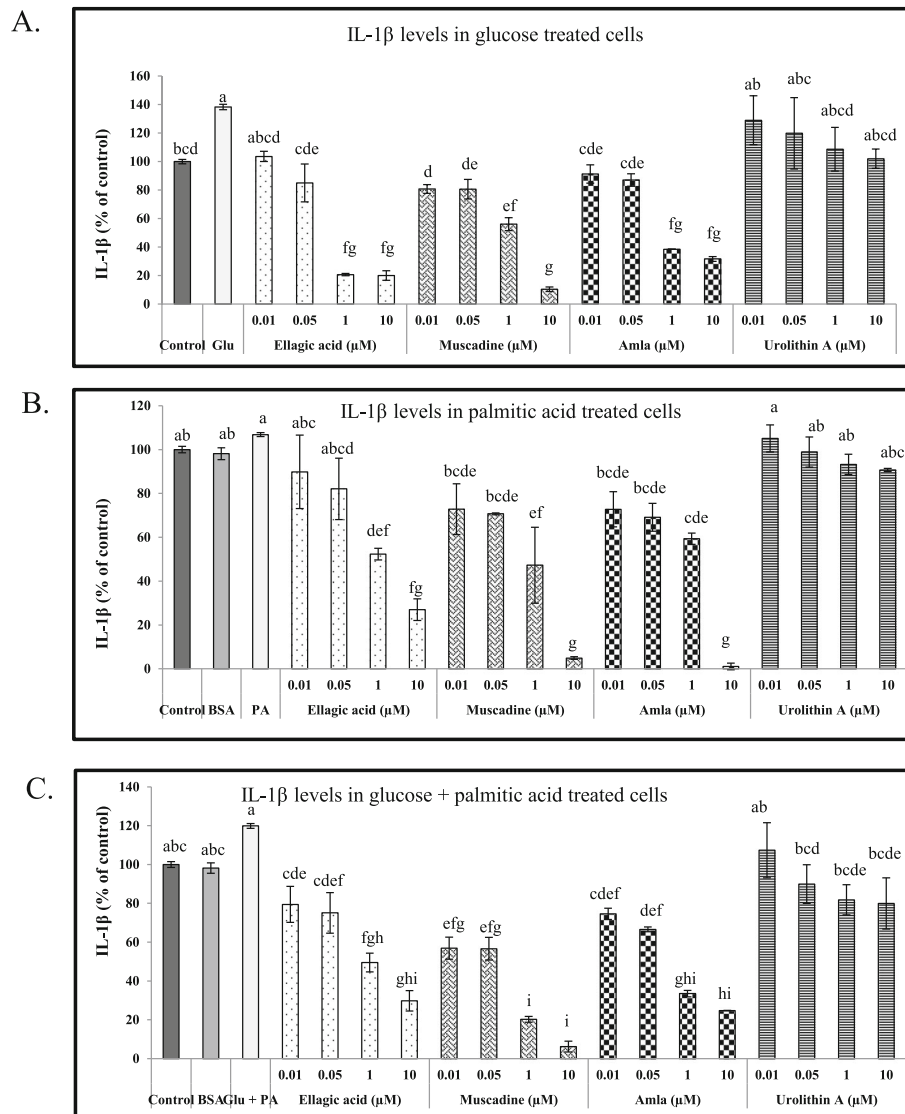To determine the effect of UR on PA-induced IL-1β, ELISA was performed and results are reported as % of

Fig. 3 a Effect of ellagic acid (EA), muscadine (MS), amla (AM), or urolithin A (UR) at 0.01-10 µM concentrations on 33.3 mM glucose (Glu)-induced IL-1β levels in NIT-1 cells. **b**. Effect of ellagic acid (EA), muscadine (MS), amla (AM), or urolithin A (UR) at 0.01-10 µM on 250 µM palmitic acid (PA)-induced IL-1β levels in NIT-1 cells. **c** Effect of ellagic acid (EA), muscadine (MS), amla (AM), or urolithin A (UR) at 0.01-10 µM concentrations on 33.3 mM glucose (Glu) + 250 µM palmitic acid (PA)-induced IL-1β levels in NIT-1 cells. The values are expressed as percentage of untreated control. Results are mean ± S. D, (n = 2). Letters with different superscripts are significantly different (P < 0.05) among groups

control IL-1β (Fig. 3b). None of the concentrations of UR tested could significantly inhibit IL-1β compared to control. Similar results were obtained from UR in Glu-treated cells (Fig. 3a). A tentative explanation of the inability of UR to inhibit IL-1β secretion may be ascribed to the presence of reduced number of hydroxyl groups in UR molecules.

Effect of EA, MS, AM or UR on Glu + PA-induced IL-1β secretion

Treatment of NIT-1 cells with a combination of Glu and PA showed a significant (P < 0.05) increase of IL-1β

levels by 19.91 ± 1.25% compared to control (Fig. 3c). The effect of EA on IL-1β induced by Glu + PA was similar to the effect observed when treated with either Glu or PA (Fig. 3a & b). All the concentrations of EA tested showed a statistically significant inhibition of IL-1β except for 0.01 µM EA. EA at 10 µM inhibited IL-1β by 70.2 ± 5.3% compared to the control. MS or AM showed significant dose-dependent inhibition of Glu + PA-induced IL-1β. Maximum inhibition of 94 and 75% compared to the control was observed with MS or AM containing 10 µM EA equivalent, respectively. Higher inhibition of IL-1β in cells treated with MS or AM

compared to EA may be the results of synergistic effect from other unidentified compounds in MS or AM along with EA. UR treatment dose-dependently inhibited IL-1β, but the inhibition was not significantly different from the control, but UR at 10 μM showed maximum inhibition by reducing IL-1β to 80 ± 13.2% of control (Fig. 3c).

Effect of EA, MS, AM or UR on insulin secretion in Glu-treated NIT-1 cells

To observe the effect of EA, MS, AM or UR on Glu-reduced insulin secretion, the supernatants from the treated NIT-1 cells were analyzed by ELISA (Fig. 4a). Glu treatment significantly ($P < 0.05$) decreased insulin secretion by 11.8 ± 0.14% of control and increased IL-1β secretion.

EA at 10 μM significantly ($P < 0.05$) stimulated insulin secretion compared to the control. The insulin concentration in cells treated with 10 μM EA was 3.8 ± 0.5% higher than that of control. EA at 0.01 μM, 0.05 μM or 1 μM dose-dependently stimulated insulin, but the difference was not significant compared to control. MS dose-dependently enhanced insulin secretion, but the increase was not significantly different from the level in Glu-treated or control cells. On the other hand, AM containing 1 μM or 10 μM equivalent of EA dose-dependently increased insulin secretion. AM containing 10 μM EA equivalent significantly stimulated insulin secretion to the level equal to that of control. From this study it can be suggested that the inhibitory effect of AM on Glu-induced IL-1β (Fig. 3a) may have contributed to enhanced insulin secretion.

To determine the effect UR on Glu-reduced insulin levels, the supernatants from the treated NIT-1 cells were analyzed by ELISA (Fig. 4a). UR dose-dependently increased insulin levels, the stimulation was not statistically significant compared to the control. A potential explanation to the low stimulatory effect on insulin in UR-treated cells is the inability of UR to inhibit Glu induced-IL-1β (Fig. 3a).

Effect of EA, MS, AM or UR on insulin levels in PA-treated NIT-1 cells

PA treatment decreased insulin level by 4.8 ± 0.3% of control (Fig. 4b). Exposure of NIT-1 cells to Glu reduced insulin secretion to a much lower level (11.8 ± 0.14%) than in the cells that were exposed to PA, compared to control. Glu induced higher levels of IL-1β secretion (38.29 ± 1.85%, Fig. 3a) compared to PA (6.84 ± 0.88%, Fig. 3b) compared to control.

Treatment with BSA alone did not have any effect on insulin secretion compared to control suggesting that the decreased insulin secretion was due to PA alone. Incubation of rat islets with PA for 48 h decreased insulin secretion by 30–50% and proinsulin biosynthesis by 30–

40% (Zhou and Grill 1994). Insulin synthesis and glucose-induced insulin secretion were inhibited when rat pancreatic islets were exposed to 250 μM PA for 48 h. Insulin content and chronic insulin secretion decreased when human pancreatic islets were exposed to 500 μM PA for 4 days (K Maedler et al. 2001).

The results in Fig. 4b show that EA dose-dependently up-regulated the insulin secretion that was reduced by PA. Among the concentrations of EA (0.01–10 μM) tested, EA at 10 μM significantly increased insulin equivalent to control untreated cells. MS or AM containing 0.01–10 μM equivalent EA dose-dependently increased insulin secretion. In the cells treated with MS or AM containing 1 μM of EA equivalent, the insulin concentration was significantly higher ($P < 0.05$) than PA and similar to the control. MS and AM at 10 μM significantly stimulated insulin secretion by 10.4 ± 0.32 and 10.6 ± 0.25%, respectively compared to control untreated cells. The synergistic effect of bioactives in MS or AM may explain their effect on insulin secretion along with EA. MS or AM are good sources of quercetin and the latter is bioavailable and may contribute to IL-1β inhibition (Cho et al. 2012).

Treatment of NIT-1 cells with UR at different concentrations (0.01–10 μM) did not stimulate insulin secretion in cells that were treated with PA (Fig. 4b). The inability of UR to inhibit IL-1β secretion (Fig. 3b) stimulate insulin secretion may be associated with its low antioxidant potential compared to EA (Landete 2011).

Effect of EA, MS, or AM or UR on insulin levels in Glu + PA-treated NIT-1 cells

Exposure of NIT-1 cells to Glu + PA inhibited insulin secretion by 10.4 ± 0.55% compared to the control (Fig. 4c). Glu + PA reduced insulin secretion more than PA (4.8 ± 0.3%) and less than Glu (11.8 ± 0.14%). Glu + PA induced more IL-1β secretion than PA alone (Fig. 3b) and less IL-1β than Glu (Fig. 3a). Chronic hyperglycemia and hyperlipidemia induce oxidative stress, an increase in IL-1β levels that interfere with the insulin signal transduction and lead to faulty insulin secretion and β-cell dysfunction (Böni-Schnetzler et al. 2008; Poitout and Robertson 2008). In this study similar inhibitory effects of GLu + PA on insulin secretion were observed.

EA dose-dependently increased insulin secretion. Nevertheless, the increase was not significantly different from Glu + PA-treated cells and lower than that of control (Fig. 4c). Treatment with MS containing 0.01-10 μM EA equivalent dose-dependently increased insulin secretion. However, the increase was not significantly different from the insulin concentration in the control (Fig. 4c). Supplementation of MS juice to diabetic patients was associated with reduced blood glucose levels and this effect may be associated with increased insulin secretion (Mitra 2007). AM treatment dose-dependently increased
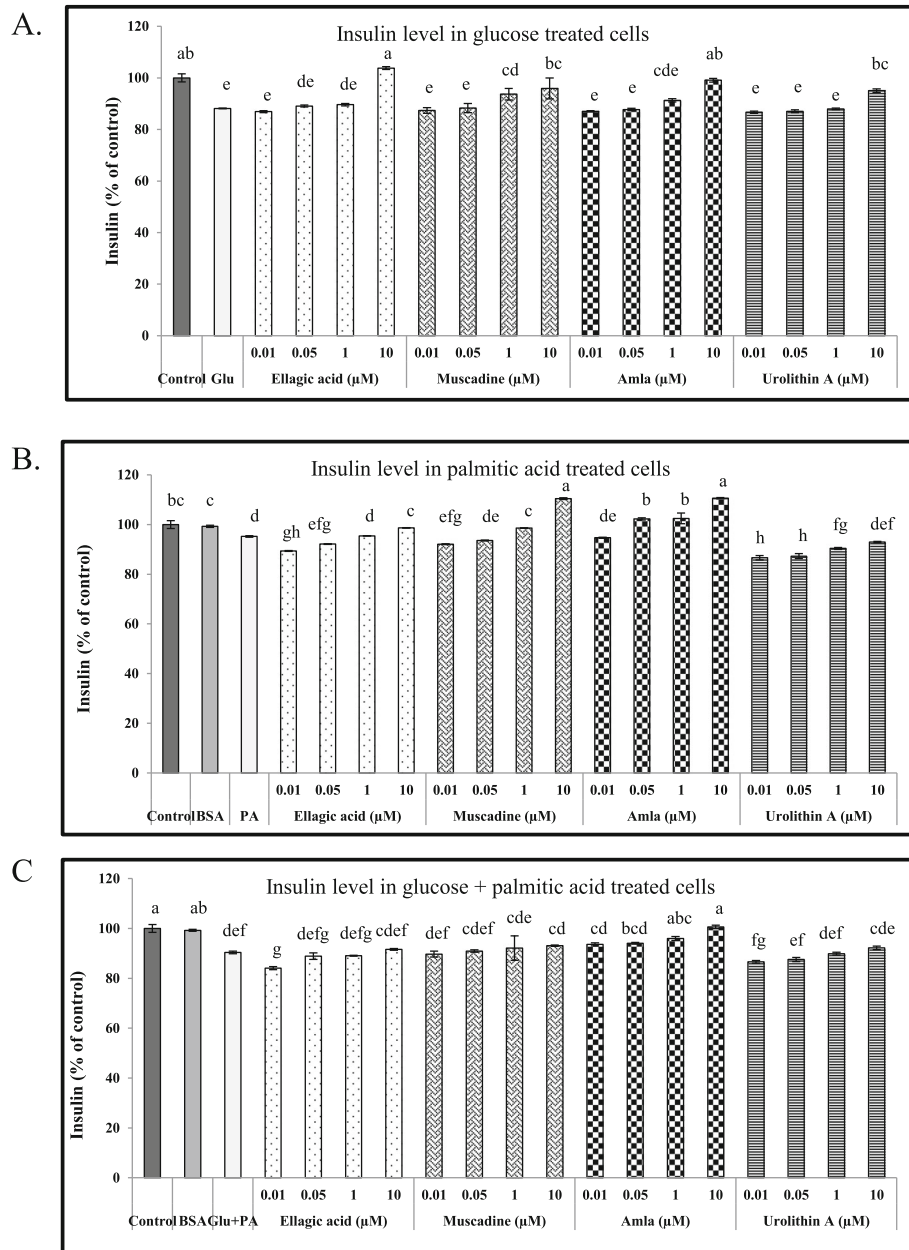
Fig. 4 a Effect of ellagic acid (EA), muscadine (MS), amla (AM), or urolithin A (UR) at 0.01-10 μM concentrations on 33.3 mM glucose (Glu)-reduced insulin levels in NIT-1 cells. **b** Effect of ellagic acid (EA), muscadine (MS), amla (AM), or urolithin A (UR) at 0.01-10 μM concentrations on 250 μM palmitic acid (PA)-reduced insulin levels in NIT-1 cells. **c** Effect of ellagic acid (EA), muscadine (MS), amla (AM), or urolithin A (UR) at 0.01-10 μM concentrations on 33.3 mM glucose (Glu) + 250 μM palmitic acid (PA)-reduced insulin levels in NIT-1 cells. The values are expressed as percentage of untreated control. Results are presented as mean ± S.D., (n = 2). Letters with different superscripts are significantly different (P < 0.05) among groups

the insulin in Glu + PA-treated NIT-1cells. However, only AM at 10 μM restored insulin similar to the level of control untreated NIT-1 cells.

UR was not effective in stimulating insulin secretion in Glu + PA-treated NIT-1 cells. There was no statistical significance between the levels of insulin secretion in Glu + PA-treated NIT-1 cells and Glu + PA + UR-treated

NIT-1 cells. Both treatments showed lower insulin secretion than the control.

Discussion

The present study investigated the effect of muscadine or amla extract standaradized to ellagic acid on the pro-inflammatory effect of glucotoxicity, lipotoxicity or

glucolipotoxicity. Ellagic acid, the major bioactive in both muscadine and amla, was used at concentrations that can be found in human plasma following consumption of ellagic acid-rich foods or beverages. Potential mechanism of the protective effect of ellagic acid- rich foods including muscadine and amla protect against diabetes was evaluated. Consumption of dealcoholized muscadine grape wine by type 2 diabetic subjects was associed with better glycemic control as shown by the lower levels of blood glucose, insulin, and glycated hemoglobin in the diabetic subjects compared to matched controls (Banini et al. 2006). The anti-inflammatory activity of muscadine was in part associated with its content of resveratrol and other polyphenols (Ghanim et al. 2011). Amla is anti-diabetic (D'Souza et al. 2014). However, the association of ellagic acid or ellagic acid-rich muscadine or amla extract and glucotoxity-, lipotoxicity- or glucolipotoxicity-associated pancreatic beta cells inflammation has never been reported. Glucotoxicity, lipotoxicity or glucolipotoxicity induces IL-1β secretion which in the presence of pancreatic β-cells mediates cell death. Exposure of NIT-1cells to hyperglycemia, palmitate or a combination of hyperglycemia and palmitate free fatty acids (FFA) increased the production and release of IL-1β, suggesting that targeting IL-1β is a logical choice in preventing β-cell apoptosis inT2DM. Blockade of IL-1β with IL-1β antibody in human with type 2 diabetes reduced inflammation and hyperglycemia, and improved β-cell function (Larsen et al. 2009). Pancreatic islets from type 2 diabetic patients exposed to palmitate release high levels of cytokines and chemokines (Ehses et al. 2007).

Exposure of NIT-1 cells to 33.3 mM glucose or 250 μM palmitic acid for 96 h significantly increased IL-1β and reduced insulin secretion. Exposure of NIT-1 cells to glucose was associated with more IL-1β secretion and insulin secretion reduction than exposure to palmitic acid. Ellagic acid, one of the major bioactives in muscadine and amla effectively inhibited the secretion of IL-1β by hyperglycemia, hyperlipidemia or a combination of both. Ellagic acid-rich muscadine or amla also inhibited IL-1β-induced apoptosis in NIT-1cells. In the present study, apoptosis was evidenced indirectly by cell viability (Fig. 2). Ellagic acid at concentration that is attainable in human plasma such as 10 μM increased cell viability and decreased β-cell apoptosis induced by IL-1β secreted by hyperglycemia, hyperlipidemia or a combination of both. Glu-, PA-, or Glu + PA- -induced IL-1β secretion was dose-dependently inhibited by EA, MS or AM standardized to its ellagic acid content. Insulin levels in Glu- or PA- treated NIT-1 cells were dose-dependently stimulated by EA, MS or AM. MS or AM stimulated insulin secretion in Glu + PA-treated NIT-1 cells. Ellagic acid did not stimulate insulin in glucose + palmitic acid treated NIT-1 cells. In muscadine or amla, ellagitannins are the sources of ellagic acid. Ellagitannins are degraded by the intestinal microflora into ellagic acid which can be absorbed as is or metabolized to urolithins (Cerda et al. 2005; Larrosa et al. 2006). The inhibition of IL-1β and stimulation of insulin by EA, or ellagic acid-rich MS or AM suggests that EA or EA-rich foods can inhibit inflammation in pancreatic β-cells and diabetes.

Conclusion
Exposure of NIT-1 cells to 33.3 mM glucose or 250 μM palmitic acid for 96 h significantly increased IL-1β and reduced insulin secretion. Exposure of NIT-1 cells to glucose was associated with more IL-1β secretion and insulin secretion reduction than exposure to palmitic acid. Glucose or palmitic acid-induced IL-1β secretion was not inhibited by urolithin A treatment. Urolithin A treatment did not stimulate insulin secretion in Glu-treated NIT-1 cells. In PA- or Glu + PA-treated NIT-1 cells, UR at 10 μM did not stimulate insulin secretion. The inability of UR in inhibiting IL-1β secretion and stimulating insulin secretion in pancreatic β-cells suggests that this metabolite may not be an effective modulator of inflammation in diabetes. In vivo studies that investigate insulin secretion following MS or AM consumption are warranted.

Authors' contributions
**JNL, SA, and SE conceived and designed the study. SE and KM acquired the data. JNL, SA, SE, MY, and KC participated in analysis and interpretation of data. SE drafted the manuscript and all authors revised the manuscript and approved the final version to be submitted.

Availability of data and materials
All data is available in the manuscript.

Competing interests
The authors declare that they have no competing interest.

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