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Effects of Oil Palm (*Elaeis guineensis*) Fruit Extracts on Insulin Secretion from BRIN BD11 Cells

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ABSTRACT

Introduction: Diabetes is one of the top ten causes of mortality in Malaysia. Several antidiabetic studies using palm oil *(Elaeis guineensis)* have been explored in recent years. The oil palm fruit itself has not been investigated and hence this study was conducted to evaluate its effects in stimulating insulin secretion from pancreatic ß-cell. **Methods:** The insulinotropic activity of oil palm fruit aqueous extract (OPF) on clonal pancreatic ß-cell line was investigated using BRIN BD11 cell line. The cell lines were incubated with different concentrations of OPF to evaluate the stimulatory effect of OPF toward insulin secretion from BRIN BD11 cells using the Rat Insulin ELISA Assay Kits. **Results:** OPF concentrations (100-1000μg/ml) were shown to significantly induce insulin secretion by a multiple of 1.97-2.58 in the BRIN BD11 cells. The highest insulin secretion increase (2.58-fold, $p<0.001$) was induced by 500 μg/ml in the OPF treated group. Evaluation of the possible mechanisms involved suggested that the mechanisms of insulin secreting activity of the 500 μg/ml OPF extract may involve the K^+_{ATP} channel-dependent pathway which exerts an insulin secretion effect through depolarising the membrane of pancreatic ß-cells. **Conclusion:** The present study has revealed the presence of insulinotropic activity in *Elaeis guineensis* fruit. Future work assessing its use as a source of active components is recommended.

Key words: *Elaeis guineensis*, hypoglycaemic, insulin, insulinotropic, oil palm fruit extract

INTRODUCTION

The insulinotropic effect or stimulation of insulin secretion from pancreatic ß-cell is an important and widely accepted mechanism by which a hypoglycaemic agent regulates hyperglycaemia (Bosenberg & Van Zyl, 2008). Sulfonylurea is the class of drugs which exerts this effect. The sulfonylurea acts by binding the ATP-dependent K^+ in the membrane cell of pancreatic ß-cells thus inhibiting the efflux of potassium. The closure of the ATP-dependent K^+channel depolarises

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the membrane and opens the voltage-gated Ca^{2+} channel causing an influx of calcium into the cells. An increased level of calcium then leads to increased fusion of insulin granule and therefore increased insulin secretion (Hannan *et al.*, 2006). To date, various types of sulfonylurea have been developed; these include repaglinide, glipizide, glibenclamide and glimepiride (Bosenberg & Van Zyl, 2008).

While sulfonylurea is being widely used for the treatment of type 2 diabetes mellitus, the side effects of the sulfonylurea drugs are well known. The drugs are reported to cause body weight gain and hypoglycaemia (David *et al.*, 2005). Due to this, much research has been directed at finding an alternative for this drug. Most of this research utilised plants as alternative sources for the evaluation of antihyperglycaemic effects (Mathews, Flatt & Abdel-Waheb, 2006; Liu *et al.*, 2009; Kalman *et al.*, 2013). Plants with high phenolic antioxidant compounds exert high potential as supplements for improving blood glucose control and preventing long-term complications among diabetics (Gallagher *et al.*, 2003). The antihyperglycemic properties of plants are reported to be associated with the polyphenolic and flavonoid content which can be found in various plants.

The oil palm fruit is planted abundantly throughout Malaysia. The fruit has been extensively researched for its health and nutritional properties, including antioxidant activities, cholesterol lowering, anticancer effects and protection against cardiovascular diseases (Sundram, Sambarthamurthi & Tan, 2003). The fruits are also reported to contain a significant degree of antioxidant polyphenolic compounds (Sundram *et al.*, 2003; Neo *et al.*, 2010). Nevertheless the antidiabetic effects of oil palm fruits on stimulating insulin secretion are yet to be known. Therefore in the present study, the ability of oil palm fruit extract (OPF) to stimulate insulin release from pancreatic ß-cell was evaluated. The BRIN BD11 cell line was used as the model of the pancreatic ß-cell.

METHODS

Sampling and sample preparation

The oil palm fruit, *E. guineensis*, was collected from the Universiti Putra Malaysia Agriculture Park. Ripe oil palm fruits were harvested from trees and used as the fresh sample. The fresh oil palm fruits were scraped into thin flakes and dried in oven overnight at 400°C. The dried fruits then were ground into small particles and the oil was removed with hexane (Merck, Germany) by using the Soxhlet method (450C, 8 h). Following removal of oil, the OPF was extracted according to the method described by Wang & Halliwell (2001). Briefly 1g of dried de-oiled oil palm mesocarp was mixed with 40 ml of 60% aqueous ethanol (Merck, Germany). Next, 5 ml of 6 M HCl (Merck, Germany) was added into the mixture prior reflux. After refluxing for 2 h, the extract was cooled, filtered, and standardised to 50 ml with 60% ethanol. Then the solvent was removed using a rotary evaporator. Finally the OPF was preserved by freeze drying.

Insulin secretion assay

BRIN BD11 cells were cultured in a cell culture flask until confluence was achieved. Once confluence was reached (80-90% confluence), the cells were seeded into a sterile 12-well plate at a concentration of 2.5 x 105 cells per well. Then the cells were incubated overnight prior to the test to allow attachment. The next day the media was removed and the cells were washed thrice with Kreb's-Ringer bicarbonate buffer (KRB). The cells were further pre-incubated for 40 min with the KRB at 37°C. After a pre-incubation period, the cells were further incubated with 1ml of KRB (negative control), 1 ml of KRB containing various concentrations of OPF extract (10-

1000 μg/ml) and 1 ml of KRB containing glibenclamide (50-1000 μM). Evaluations were performed at 2mM glucose. After incubation the aliquots from each well were transferred into 1.5 ml tubes. Finally the aliquots were stored at -20^{O}C for insulin assay using a rat insulin ELISA kit (LINCO Research, USA).

Determination of insulin concentration

The insulin secreted from BRIN BD11 cell line was measured using Rat Insulin ELISA Assay Kits (LINCO Research, USA). First the antibody-coated 96-well plate were washed trice with 300 μl wash buffer. Then 10 μl of assay buffer, standards, controls and samples were added into the well plate respectively. Next 80 μl of detection antibody was added into each well and the plates were sealed and incubated for 2 h at room temperature. After the incubation period, the plates were washed thrice, again with 300 μl wash buffer. Then 100 μl of enzyme solution was added into each well and the plate was sealed and further incubated for 30 min at room temperature. Following the required incubation period, the plate was washed 6 times with 300 μl wash buffer. Next 100 μl of substrate was added into each well and the plate was sealed and further incubated for 15 min at room temperature under a dark condition. The change in colour from yellow to blue (indicating the presence of insulin) in each well was observed during the incubation period. Finally 100 μl of stop solution was added to stop the reaction and the absorbance was measured at 450 nm using a micro plate reader (Mindray MR-96A, Shenzen).

Determination of insulin secretion mechanisms

The extract that showed the most tremendous stimulation of insulin secretion from the BRIN BD11 cell (500 μg/ml OPF) was selected to evaluate the possible mechanisms underlying the insulin secretory action of oil palm extracts. Various modulators were used which are known to affect the secondary messenger pathway in pancreatic ß-cell. First, the cells were seeded into a sterile 12-well plate at a concentration of 2.5 x 10^5 cells per well. Then the cells were incubated overnight prior to the test to allow attachment. The next day the media was removed and the cells were washed thrice with KRB. The cells were further pre-incubated for 40 min with the KRB at 37^{O}C. After a pre-incubation period, the cells were further incubated with 1ml of KRB containing 500 μg/ml OPF extract in the presence and absence of isobutylmethyxanthine (IBMX) and tolbutamide (insulin secretagouge), diazoxide and verapamil (insulin secretion inhibitor) and potassium chloride (KCl) at a concentration of 100 μM, 200 μM, 300 μM, 50 mM and 30 mM, respectively. The evaluation was performed at 2 mM glucose. After incubation the aliquots from each well were transferred into 1.5 ml tubes. Finally the aliquots were stored at -20^{O}C for insulin assay.

Statistical analysis

Data were expressed as mean ± standard deviation. One-way ANOVA (GraphPad Prism 5) were used for analysis and groups were considered significantly different at the 5% significance level (p<0.05). Dunnet post-hoc test was done for a significant value obtained from ANOVA.

RESULTS AND DISCUSSION

The mechanism of insulinotropic agents to stimulate insulin secretion from pancreatic ß-cells involving K^+_{ATP} channel-dependent pathway is well known. The agents act by binding the ATP-dependent K^+ in the membrane cell of pancreatic ß-cells thus inhibiting efflux of potassium. The closure of ATP-dependent K^+channel depolarises the membrane and opens the voltage-gated Ca^{2+} channel causing an influx of calcium into the cells. An

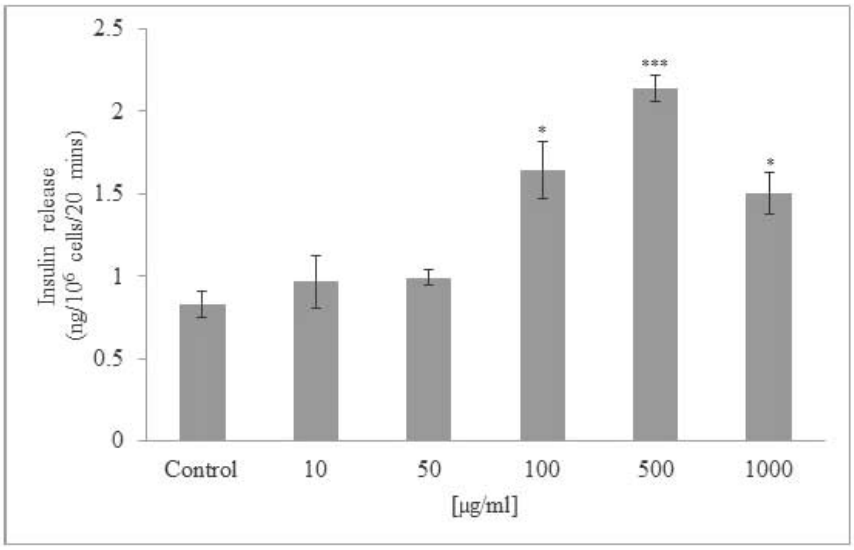

Figure 1. Effect of OPF extract on insulin secretion from BRIN BD11 cells. Values represent the means ± S.D. *$p<0.05$ and ***$p<0.001$ compared with control

increased level of calcium then leads to increased fusion of the insulin granule and therefore increases insulin secretion (Hannan *et al.*, 2006). Nevertheless there is also another pathway which is known to stimulate insulin release. It is the K^+_{ATP} channel-independent pathway. Under this condition, which activates by cAMP, the glucose does not increase the intracellular Ca^{2+} concentration but amplifies the Ca^{2+} action on the releasing process (Hiroki *et al.*, 1999).

In the present study the BRIN BD11 cell line was used to evaluate the effect of OPF extract on insulin secretion. The OPF was observed to significantly stimulate insulin secretion from BRIN BD11 cells at certain concentrations (Figure 1). This result suggests that there is a possibility that antidiabetic compounds are present in the extract which exerts its antihyperglycemic effect through stimulation of insulin secretion from the pancreatic *ß*-cells. The stimulation of insulin from BRIN BD11 cells was significantly observed at 100 µg/ml treatment group, 1.97-fold ($p<0.05$) followed by the 500 µg/ml treatment group which enhanced insulin secretion the most by 2.58-fold ($p<0.01$) of stimulation relative to the control group. However, the result was not concentration-dependent since the insulin secreted at a higher OPF concentration (1000 µg/ml) was lower compared to the 500 µg/ml OPF concentrations. This may be due to the result of 1000 µg/ml OPF that reduced the viability of BRIN BD11 cells by less than 50% in the viability assay (data not shown). The high OPF concentration might affect the physiological function of BRIN BD11 as result of the presence of toxic compounds at such concentrations, hence reducing the insulin secreted.

In the present study, the glibenclamide was used to challenge the effect of OPF extract to stimulate insulin secretion from BRIN BD11 cells. The 1000 µM of glibenclamide was selected as a positive control since this concentration produces the highest stimulation of insulin release after a dose-dependent evaluation (Figure 2). Nevertheless the stimulation of insulin secretion by OPF extract was lower compared to 1000 µM glibenclamide

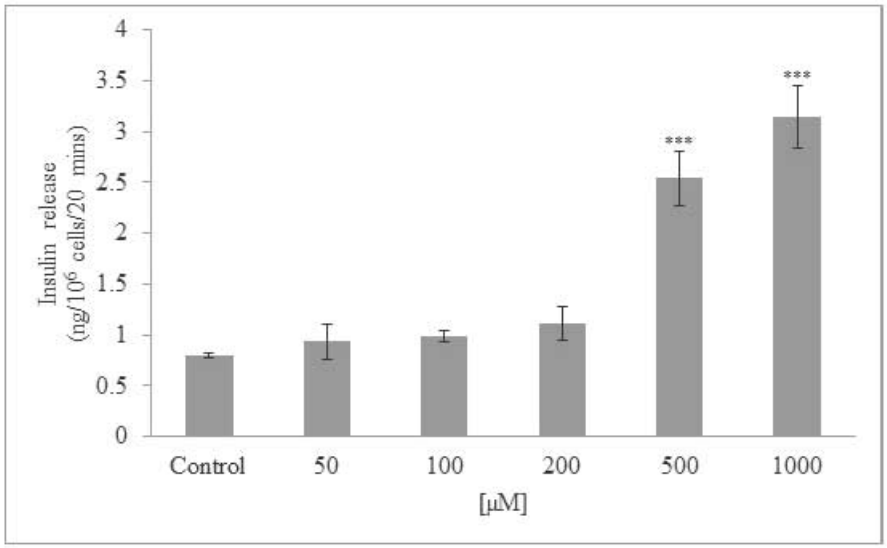

Figure 2. Effect of glibenclamide on insulin secretion from BRIN BD11 cells. Values represent the means ± S.D. ***p<0.001 compared with control

which stimulates insulin secretion from BRIN BD11 cells by 3.92-fold (p<0.001). This could be due to the fact that the OPF extract contains a mixture of bioactive and non-bioactive compounds. When the non-bioactive compounds in the extracts are interrupted, the concentration of active compounds is reduced which in turn reduces the extract's ability to stimulate insulin secretion from BRIN BD11 cells. Compared to the extract, the glybenclamide consists of a single and pure compound, hence increasing its effectiveness. Furthermore, the potential of glybenclamide to stimulate insulin secretion from the pancreatic β-cells has also been scientifically proven (Irwin *et al.*, 2010).

For evaluation on the insulin secreting mechanisms, the extract was incubated with various insulin modulators to better understand the mechanisms underlying the insulin secretory action of the oil palm extract. The 500 μg/ml OPF was selected in the experiment as the extract at such a concentration to show the most potent insulin secretion activity from BRIN BD11 cells (Figure 1). The insulin modulators used were from the insulin secretagoue group such as IBMX and tolbutamide, insulin inhibitors such as diazoxide and verapamil as well as the depolarising agent KCl at a depolarising concentration of 30 mM. Tolbutamide, which is from the sulfonylurea group, exerts its insulin secreting mechanisms by closing the K^+_{ATP} channel, depolarising the plasma membrane, and stimulating the influx of Ca^{+2} through the activation of the voltage-dependent calcium channel (Kecskemati *et al.*, 2002). Therefore, tolbutamide is a K^+_{ATP} channel-dependent modulator. IBMX, on the other hand, is a K^+_{ATP} channel-independent modulator. It is a non-selective cyclic adenosine monophosphate (cAMP) phosphodiesterase inhibitor which raises concentrations of cAMP in pancreatic β-cells. The increased concentration of cAMP activates the insulin secretion process of pancreatic β-cells (Hiroki *et al.*, 1999).

In this study, the presence of tolbutamide significantly enhanced the insulin secretion activity of 500 μg/ml OPF extract (Figure 3). However, in the

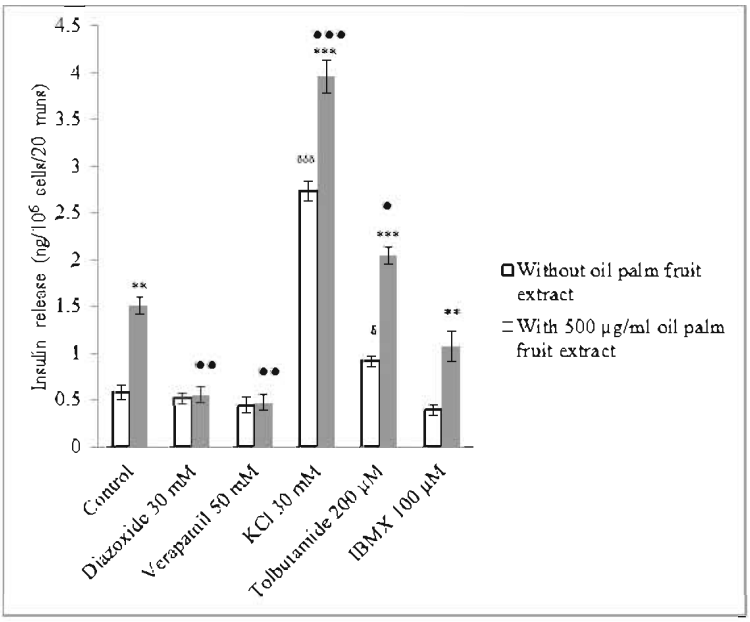

Figure 3. Effect of various insulin secretion modulators on the insulin secretion activity of 500 μg/ml OPF from BRIN BD11 cells.

Note:s Values represent the means ± S.D. $^{**}p<0.01$ and $^{***}p<0.001$ compared with control without 500 μg/ml OPF extract in respective group. $\bullet p<0.05$, $\bullet\bullet p<0.01$ and $\bullet\bullet\bullet p<0.001$ compared with the 500 μg/ml OPF extract. $^{\delta}p<0.05$ and $^{\delta\delta\delta}p<0.001$ compared to untreated secretion.

presence of IBMX, there are no significant changes in the insulin secreting activity of the extract. This result suggess that the mechanisms of insulin secreting activity of the 500 μg/ml OPF extract may involve the K^+_{ATP} channel-dependent pathway which exerts an insulin secretion effect through depolarising the membrane of pancreatic β-cells and not through the K^+_{ATP} channel-independent pathway which mediates insulin secretion through protein kinase A activated by cAMP (Hannan *et al.*, 2007).

Diazoxide is an insulin inhibitor which inhibits glucose-induced insulin secretion by opening the K^+_{ATP} dependent-channel. The opened gated K^+_{ATP} prevents the membrane depolarisation, thus inhibiting the influx of Ca^{2+} into the pancreatic β-cells (George & McCrimmon, 2012). Alternatively, the insulin inhibitor, verapamil, inhibits insulin by blocking the voltage-dependent Ca^{2+} channel, thereby preventing the influx of Ca^{2+} into the pancreatic β-cells. In depolarising conditions, the closing of voltage-dependent Ca^{2+} prevents the influx of extracellular Ca^{2+} thus inhibited the insulin release that depends on voltage-dependent Ca^{2+} channel activation (Yousef *et al.*, 2005). In the present study both diazoxide and verapamil were observed to inhibit the insulin secretion activity of the 500 μg/ml OPF extract. Therefore, from the results it can be suggested that the effect of insulin release was Ca^{2+} dependent, which is partly acquired from the extracellular through the activation of the voltage-dependent Ca^{2+} channel and allowing for the influx of Ca^{2+} (Hannan *et al.*, 2006). Nevertheless to prove this suggestion, further studies are required to isolate and purify the bioactive compounds to be used in the insulin secreting mechanisms evaluation of the extracts.

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