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Research Article

Momordica dioica Stimulated Insulin Secretion from Rat Pancreatic Islets is Independent of ATP-Sensitive K^+ Channel

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ABSTRACT
Antidiabetic and insulin secretagogues activity of *Momordica dioica* has been reported earlier. In the present work, the effect of Aqueous Extract of *Momordica dioica* (AEMD) was studied on insulin secretion with an evaluation of its mechanisms of action. Phytochemical analysis of AEMD was also carried out. AEMD showed the presence of alkaloids, saponins, flavonoids, tannins, and sugars. Isolated pancreatic islets of normal Wistar rats were incubated in HBBS buffer at 3.3 and 16.7mM glucose, 1 and 2mg/ml AEMD and 1mg/ml of nicorandil, alone and in combination. Release of insulin in external media was measured by ELISA. AEMD stimulated insulin release from the isolated islets at 3.3 and 16.7mM glucose. Insulin release was more pronounced at 2mg as compared to 1mg concentration. AEMD mediated enhancement in insulin secretion was not diminished by nicorandil, suggesting K-ATP channels independent mechanism. Cytotoxicity studies were carried out by trypan blue uptake and LDH release assay. Trypan blue gained access to 8.2±1.9% cells while 7.1± 1.4% dead islet cells were observed in LDH release assay on AEMD exposure, proving that the extract was non toxic at tested concentration. AEMD released insulin in a dose dependent manner and this induction was independent of K-ATP channels of β-cells.

Key words: *Momordica dioica*, Phytochemical analysis, insulin; K-ATP channels, nicorandil

INTRODUCTION

Impaired insulin release is major problem associated with diabetes mellitus. Drugs enhancing insulin release from the pancreatric β-cells are widely used for management of type-1 as well as type-2 diabetes[1]. ATP-sensitive K^+ (K-ATP) dependent[2] and K-ATP channel-independent[3] pathways have been proposed for insulin release form β cells. In the K-ATP channel-dependent pathway, enhanced glucose metabolism increases the cellular ATP/ADP ratio leading to closure of K-ATP channels, depolarization of cell membrane and activation of the voltage-dependent L-type Ca^{2+} channels. The activation of Ca^{2+} channels increases Ca^{2+} entry in the cell [4] which stimulates insulin release[5].

A number of insulin secretagogues agents (sulfonylureas, incertins etc.) are currently used for management of diabetes[6]. Sulfonylureas are widely used mainly because of their low costs as compared to other secretagogues but long term use of sulfonylureas results in β-cell exhaustation and treatment failure. Hence there is need for relatively safer and cost effective secretagogues. Medicinal plants have been used for management of diabetes. A number of these plants have been reported to possess insulin secretagogues activity [7,8]. The genus Momordica is well known for its antidiabetic activity [9]. Antidiabetic activity of *Momordica dioica* has been previously reported by our group with significant increase in serum insulin level [10].The present study was designed to investigate the mechanism of *M. dioica* induced insulin release from isolated islets of healthy Wistar rats. The phytochemical analysis of the *M.dioica* was also carried out to indentify the active molecule(s).

MATERIALS AND METHODS

Plant material

Fruits of *M. dioica* were collected from local hills of Bundelkhand University during August- September, 2010. The material was identified with the help of experts in Department of Botany and a voucher specimen no. BU/BMS/VS/2010/03 was preserved in Department of Biomedical Sciences, Bundelkhand University. The fruits were cut in small pieces, shade dried and grinded to obtain coarse powder. The powder (1kg) was soaked overnight in distilled water in a ratio of 1gm to 3 ml with constant stirring. The extract was filtered through Whatman No. 1 filter paper and centrifuged at 10,000 rpm for 10 min to remove any residual material. The supernatant was lyophilized to obtain aqueous extract of *M. dioica* (AEMD). The extract was stored at -20ºC until use.

Phytochemical analysis of AEMD

Phytochemical analysis for alkaloids, saponins, flavonoids, tannins, terpenoids, sugars, anthaquinones, phalobotanins and cardiac glycosides of AEMD was performed for as per the methods of Sofowara [11].

Isolation of pancreatic islets

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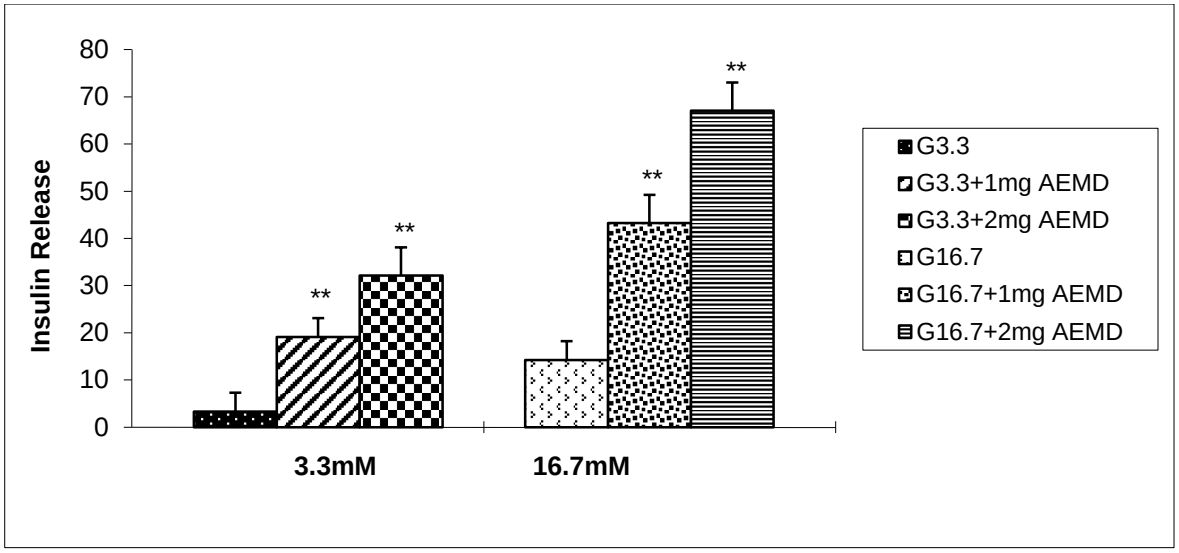

Figure 1: Dose dependent effect of AEMD on insulin release at 3.3 and 16.7mM glucose. Each bar expressed as Mean ± SEM for 6 observations. ** =P<0.01 compared with control.

Table 1: Effects of glucose, AEMD and nicorandil on insulin release from isolated islets.

HBBS, BSA, Islet and Addition to the medium	Concentration of AEMD and Nicorandil (1mg/ml) Glucose (mM)	
	3.3	16.7
None	3.3±0.071	14.4±0.096
Nicorandil	3.1±0.065•	4.73±0.049**
AEMD	18.275±0.04**	44.46±0.075**
AEMD+Nicorandil	16.775±0.11**	30.48±0.081**

The value represents as Mean± SEM for 6 observations. •=p>0.05, ** =p<0.01 compared with control (None, No addition in the medium)

Pancreas was excised from healthy Wistar rats of either sex and islets were isolated by the method of Lacy and Kostianovsky [12]. The use of animals was approved by Institutional Animal Ethical Committee (IAEC) vide proposal reference no. BU/Pharm/IAEC/11/038 dated 26.03.2012. IAEC was constituted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India.

Insulin secretion and measurement

To study the effect of glucose and AEMD on insulin release, batches of 4-5 islets were incubated in HBBS (5% CO_2, pH 7 .4) containing 1% BSA, 3.3 or 16 .7 mM glucose and 1or 2 mg/ml of AEMD. To investigate the mechanism of insulin release, islets were incubated with nicorandil (1mg/ml) in presence or absence of glucose and AEMD (1mg/ml). After 1 hr, the incubation mixtures were centrifuged at 16000g, the supernatants were collected and used for insulin measurement by enzyme-linked immune-sorbent assay kit (DRG Instruments GmbH, Germany).

Cell viability

Cell viability test on isolated islets was performed by trypan blue and lactate dehydrogenase (LDH) release assay. In trypan blue assay, the islets cells were prepared as described previously [13] and were exposed to trypan blue (0.1%w/v) for 1 hr at 37^0C. The presence of dye was determined by light microscopy to calculate the percentage of live and dead cells [14]. For LDH measurement batches of 100 pancreatic islets were incubated with AEMD (2mg/ml) and nicorandil (1mg/ml) for 1 hr at 37^0C. The leakage of LDH in the supernatant was measured using automated clinical chemistry analyzer VITALAB Selectra_E, following kit instructions (Merck Specialist Pvt. Ltd) at 390nm with necessary modifications [14,15]. The percentage of cytotoxicity was calculated by following equation,

Cytotoxicity (%)=(Experimental value -Low control value) x100/ (High control value- Low control value)

Whereas, experimental value was LDH activity of islets incubated with AEMD and nicorandil, low control value was islets incubated in HBBS buffer and high control value was islets incubated with 1% (v/v) Triton X-100.

Statistical analysis

Results were expressed as Mean ± SEM for 6 animals in each group. Hypothesis testing method included one-way analysis of various (ANOVA) followed by Dunnette's comparison tests. P-values of less than 0.05 were considered to indicate statistical significance. All the statistics were carried out in GraphPad InStat Software Inc., v. 3.06, San Digeo, USA.

RESULTS

Phytochemical analysis of AEMD

Phytochemical analysis of AEMD showed the presence of alkaloids, saponins, flavonoids, tannins, and sugars.

Effect of AEMD on insulin release

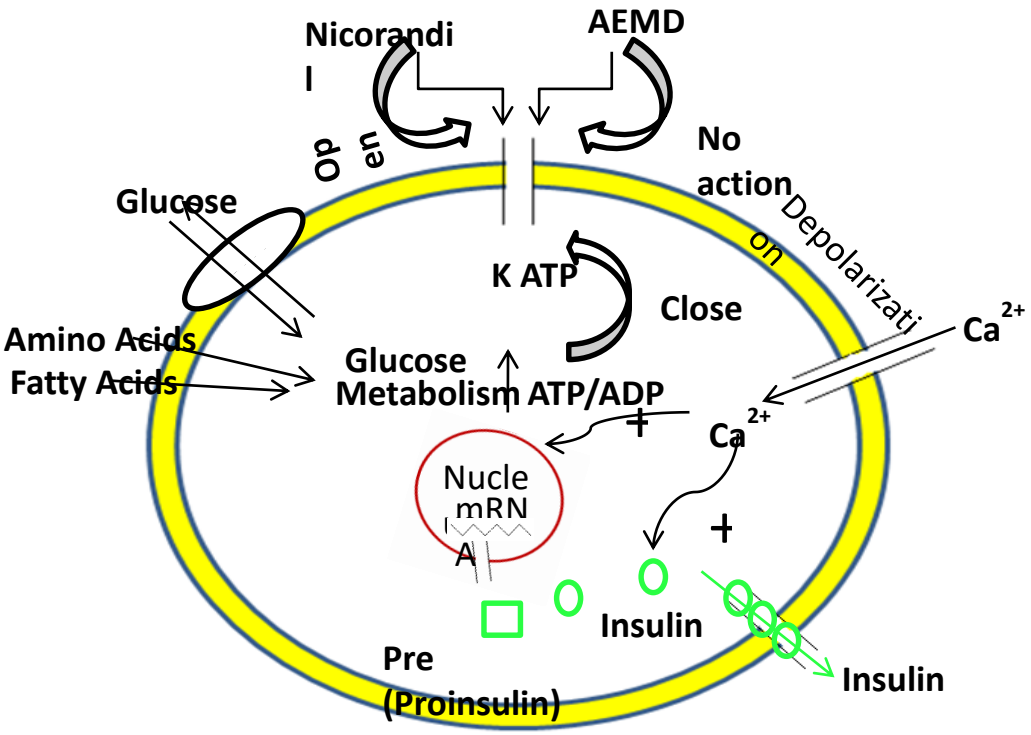

Figure 2: Effect of AEMD and nicorandil on insulin secretion from β-cells

Exposure of isolated islets to 16.7mM glucose stimulated insulin release as compared to basal insulin level at 3.3mM glucose concentration, confirming the sensitivity of the assay system. AEMD (1mg/ml) stimulated insulin secretion from 3.3±0.126 µU/islet/1hr to 19.115±0.055 at 3.3 mM glucose and 14.191±0.096 to 43.258±0.116 to at 16.7 mM glucose. At 2mg/ml concentration of AEMD, insulin level was 32.130 ±0.068 and 67.064±0.165 at 3.3 and 16.7mM glucose respectively. The results indicated that insulin secretagogues effect of AEMD was dose dependent (Fig. 1).

Effects of AEMD and nicorandil on insulin release from isolated islets

Nicorandil significantly ($p<0.01$) decreased glucose stimulated insulin release at 16.7mM of glucose, however no significant ($p>0.05$) change was observed at basal insulin level at 3.3mM of glucose. Nicorandil reduced AEMD (1mg/ml) induced insulin secretion at 16.7mM by 31% ($p<0.01$) where as only 8.2% ($p<0.01$) reduction was observed at 3.3mM glucose (Table 1).

Cell viability assay

Trypan blue gained access to 7.5±1.3% and 8.2±1.9% islet cells respectively, at 1 and 2 mg/ml concentration of AEMD and 3.2±1.8% cells on nicorandil (1mg/ml) exposure for 1 hr. In LDH release assay, the percentage of dead islet cells after 1 hr exposure was 6.9± 1.7% and 7.1± 1.4% respectively at 1 and 2 mg/ml concentration of AEMD and 3.1±1.2% at 1mg/ml nicorandil. The study indicated that AEMD and nicorandil were non toxic at tested concentrations.

DISCUSSION

Various species of the genus Momordica are reported to possess antidiabetic activity. Antidiabetic activity of *Momordica dioica* with increase in serum insulin has been previously reported by our group [10]. Hence, this study was carried out to evaluate the mechanism of insulin secretion by AEMD. Isolated pancreatic islets serve as a fast and cost effective *in vitro* model for studying insulin release [16]. In present study, AEMD stimulated insulin release form isolated islets in dose dependant manner with 2mg/ml concentration showing high release as compared to 1mg/ml. The effect at higher dose was not cumulative possibly due to other limiting factors like concentration of Ca^{+} or exocytosis of insulin to near maximal. Paesuad *et al.* reported that loss of islet cells membrane integrity was responsible for increase in insulin release by saponins isolated from *Gymnema sylvestre* [17] Alteration in islets cell membrane permeability by AEMD may be a possible mechanism of insulin release in present study also. AEMD treatment of islets for 1 hr did not increase trypan blue uptake, indicating that membrane integrity was not alerted. The findings were further supported by LDH release assay where only 7.1± 1.4% dead islets cells were reported on AEMD (2mg/ml) treatment. Hence, the possibility of alteration of membrane integrity by AEMD as possible mechanism of insulin release is ruled out by this study. The other well known physiological mechanism for insulin release involves K-ATP and voltagegated Ca^{2+} channels present on β-cells. The common physiological stimulant, glucose exerts its insulinotropic action by increasing cellular level of ATP, causing closure of K-ATP channels, leading to depolarization of β-cell membrane and increase in Ca^{2+} influx via the Ca^{2+} channels. This Ca^{2+} influx stimulates release of insulin by exocytosis [18]. To explore this possibility of insulin release, nicorandil (a K-ATP channel opener) was used along with AEMD. Nicorandil binds to K-ATP channels, hindering their ATP mediated

closure subsequent membrane depolarization and opening of Ca^{2+} channels. Addition of nicorandil completely abolished glucose stimulated insulin secretion at 16.7mM glucose, indicating its K-ATP binding capability and insulin secretion inhibitory activity. Addition of AEMD increased insulin secretion at 3.3 and 16.7mM of glucose, indicating its insulin secretogogues potential. When islets were incubated with AEMD and nicorandil, AEMD induced insulin release at 16.7mM was decreased but not abolished completely. However, no change in insulin secretion was observed at basal glucose level of 3.3mM. No change in insulin secretion at basal level of glucose may be due to non binding of phytochemicals of AEMD to K-ATP channels. It is possible that after addition of nicorandil with AEMD at 16.7mM of glucose, the partial decrease in insulin secretion may be due nicorandil mediated effect. The results indicated that AEMD induced insulin secretion is independent of K-ATP channels of β-cells (Fig. 2). The possible targets of AEMD may be posterior to the K-ATP channels and further work is required to explore these targets. Phytochemical analysis of AEMD showed the presence of alkaloids, saponins, flavonoids, tannins and sugars. AEMD may be explored further for purification and identification of molecule(s) responsible of insulin secretogogues activity.

CONCLUSIONS

The results indicated that AEMD induced insulin secretion is independent of K-ATP channels of β-cells. Further work is required to explore the mechanism of AEMD induced insulin release.

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CONFLICT OF INTEREST

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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