Original Article

The reparative effects of *Momordica Charantia Linn.* extract on HIT-T15 pancreatic β-Cells

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The aim of this study is to investigate the cell reparative effects of *Mormordical Charantia Linn.* boiling water extract (MCE) on the HIT-T15 Hamster Pancreatic β- cells. Furthermore, the superoxide dismutase (SOD) activity of MCE was determined. 0.02% MCE (w/v) achieved the highest cell proliferation rate of 45.6% (p<0.01) on alloxan damaged HIT-T15 cells while 0.2% MCE increased the proliferation of the normal cells by 35.4% (p<0.05). The high molecular weight fraction of MCE (MHMF, MW>3 kDa) showed the stronger effects in repairing alloxan damaged cells (cell proliferation rate=32.1%, p<0.05) than that of the low molecular weight fraction (MLMF, MW≤3 kDa), while the latter showed the higher activity on increasing insulin secretion of normal or damaged cells. 2%MCE and MLMF showed the highest SOD activities, 19.74 NU/mL and 19.84 NU/mL, but they failed to improve the proliferation rate of alloxan damaged cells. These results indicated MCE has significant repairing effects on HIT-T15 cells against superoxide anion radicals, which did not correlate to MCE's SOD activity. It was hypothesized that the different fractions of MCE may make different contributions to MCE's cell repairing activity and its ability of stimulating insulin secretion.

Key Words: Cellular Repair, *Momordica Charantia Linn.* Extract, HIT-T15 Pancreatic β-Cells

Introduction

Diabetes mellitus is a growing threat to public health in modern society. Bitter gourd (*Momordica Charantia Linn.*) is one of the frequently used antidiabetic herbs in China and Southeast Asia. Its hypoglycemic activity has been reported in pulps, seeds and leaves *in vivo*.[1] Bitter gourd fruit juice can stimulate glucose uptake of skeletal muscle cells.[2] The previous studies mainly focused on the active ingredients within the fresh bitter gourd. Khanna *et al*. isolated an active protein (*p*-insulin) from seeds by acid-ethanol extraction,[3] which decreased blood glucose in STZ-induced diabetic rats and increased the glycolytic enzymes activity. Proteins from bitter gourd were proven to stimulate the insulin secretion and glucose uptake in rat myocytes and adipocytes.[4] Other effective compounds, such as charantins and alkaloids[-5] have also been identified from fresh bitter gourd.

The boiling water extract of *Momordica Charantia Linn.* (MCE) has been proven to be a potent anti-diabetic agent and has been applied clinically to both Type 1 and Type 2 diabetes patients.[6,7] The effective ingredients within MCE and their pharmacological mechanism, which may differ from those of the fresh bitter gourd, have rarely been investigated and are little understood.

The present study aimed to investigate the therapeutic effects of MCE by determining the cell reparative effects of MCE on HIT-T15 Hamster pancreatic β-cells against alloxan damages and the influence of MCE on insulin secretion. Furthermore, the superoxide dismutase (SOD) activity of MCE was determined.

Materials and methods

Materials

Alloxan, tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenylte- trazolium bromide) and Dimethyl sulfoxide (DMSO) was purchased from Sigma chemical. HEPES and RPMI1640 culture medium were purchased from Gibco Industries, Inc. Trypsin was purchased from Xiamen Tagene Biotechnology Inc. Insulin radioimmunoassay (RIA) Kit was purchased from Tianjin Nine Tripods Medical Bioengineering Co., Ltd. FBS and ES was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. Superoxide Dismutase (SOD) Detection Kit were purchased from Nanjing Jiancheng Bioengineering Institute. High quality, standardised fresh fruits of bitter gourd and sliced sun-dried pulps were purchased from the farm in Huxian county, Shanxi province, P.R. China.

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The boiling water extracts (100°C) of sun-dried pulps of bitter gourd were vacuum concentrated at 70 °C to get a brown powder of MCE. The fresh juice of 500 g fresh pulp of bitter gourd was squeezed using a food processor for 10 min and filtered with filter paper and 0.45 μm acetyl cellulose membrane. 140 g sun-dried bitter gourd was extracted by 700 mL cold water for 24 hr at 4°C and then filtered and vacuum concentrated at 35°C to 100 mL (correspond with 20%). 50 ml 20% MCE (w/v) was dialyzed with dialyzer (MW=3000 Dalton) in 500 mL deionized water for 24 hr to prepare the MCE high molecular weight fraction (MHMF) and the MCE low molecular weight fraction (MLMF). The solvents of MHMF and MLMF were vacuum concentrated at 35 °C to about 50 mL, respectively.

Cell culture

HIT-T15 were maintained in RPMI 1640 and supplemented with 10% FBS and 5% ES at 37 °C (5% CO_2 in air). Cells in culture flask were digested with Trypsin. Cells were transferred to 96-well plates (5×10^5 cells/mL) and incubated for 24 hr, prior to mixing with alloxan (0.1 mmol/L in PBS) at 37 °C for 1 hr. After removed the alloxan solvent, the culture medium with serial diluted MCE and other bitter gourd preparations were added and incubated for further 24 hr. The PBS was added instead of bitter gourd samples as control.

The proliferation rate assay

The proliferation rate of HIT-T15 cells was determined by MTT assay[8] and calculated with the following formula: Cell Proliferation Rate (CPR) % = ($OD590_{\text{sample incubated}}$ - $OD590_{\text{control}}$) / $OD590_{\text{control}}$ ×100

SOD activity assay

The SOD activity of MCE was determined by xanthine oxidase assay.[9]

Insulin concentration assay

Insulin concentration in the cells' cultivation supernatant was measured by radioimmuno assay (RIA).[10] The change rate of insulin secretion was calculated with the following formula: RIA Rate % = (RIA $\text{Value}_{\text{sample incubated}}$ − RIA $\text{Value}_{\text{control}}$) / RIA $\text{Value}_{\text{control}}$ ×100.

Data expression and statistical analysis

Data were expressed as the mean ±S.E.. Data were analyzed by *t*- test. The significance level was set at $p<0.05$.

Results and discussion

The proliferation rate and the amount of insulin secretion of HIT-T15 cells were determined, in order to evaluate the effects of three kinds of bitter gourd preparations and two MCE fractions on the normal pancreatic β- cells *in vitro*. As described in the Fig 1, MCE, totally inhibited the cell growth at 2% (w/v) but significantly increased the cell proliferation rate by 35.5% at 0.2% (w/v, $p<0.05$). Other bitter gourd preparations employed in this study, including the MCE at 0.02% (w/v), did not show significant activity on the cell growth.

Except 2% MCE and the high molecular weight fraction of MCE (MHMF), all of others increased the insulin

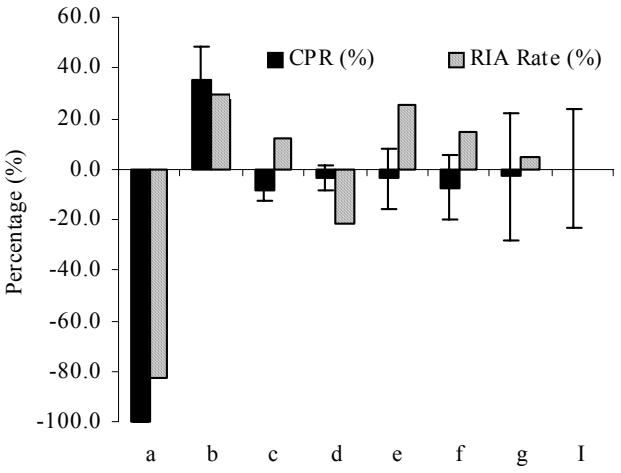

Figure 1. Effects of the bitter gourd preparations on the proliferation and insulin secretion of normal HIT-T15 Cells
a. 2% MCE, b. 0.2% MCE, c. 0.02% MCE, d. 2%MHMF, e. 2%MLMF, f. juice of fresh bitter gourd pulps, g. cold water extract of sun-dried bitter gourd, i. normal cells control. CPR: Cell Proliferation Rate; RIA Rate: the percentage of insulin secretion changes. *: $p<0.05$, n=4.

secretion of HIT-T15 cells by various rates. The 0.2% MCE stimulated the insulin secretion on a high value of 318.96 μIU/mL and the low molecular weight fraction of MCE (MLMF) obtained that of 309.26 μIU/mL, while normal cells secreted 246.40 μIU/mL during the 24 hours incubation. It appears that the increasing percentage (29.4%) of the insulin secretion on the 0.2% MCE incubated cells corresponded with the growth on their cell proliferation rate (35.5%). On the contrary, 2% MLMF showed its activity in promoting the β- cells' insulin secretion and no influence on the cell proliferation.

Since the plant insulin-like-polypeptide of bitter gourd was reported,[3] the insulin radioimmunological reactivity of MCE was determined with radioimmunoassay. According to Table 1 and Fig 2, the RIA values of MCE were concentration dependent. The insulin RIA value of 0.2% MCE was 1.92 μIU/mL (shown in Table 1) and much lower than the 72.56 μIU/mL measured on the HIT-T15 cells incubated with the same sample. The significant difference on insulin RIA value indicated that 0.2% MCE promoted the insulin production or secretion of HIT-T15 cells.

Neither the growth nor the insulin secretion of HIT-T15 cells was inhibited by the bitter gourd preparations, besides the MCE at extraordinary high concentration (2%). The bitter gourd preparations, therefore, had the very low cytotoxicity on HIT-T15 cells and increased the insulin secretion slightly.

Table 1. The insulin radioimmuno assay of MCE

Concentration of MCE (%, w/v)	RIA of MCE (μIU/mL)
20	57.71
5	13.42
2	5.24
0.20	1.92
0.020	0.26

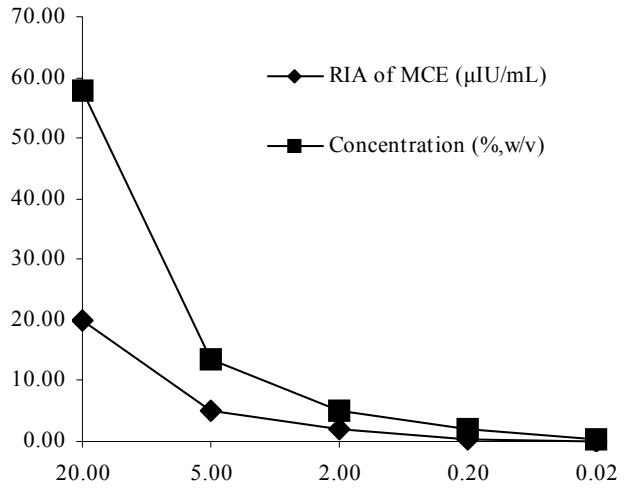

Figure 2. The relation of MCE's insulin immunological activity and its concentration.

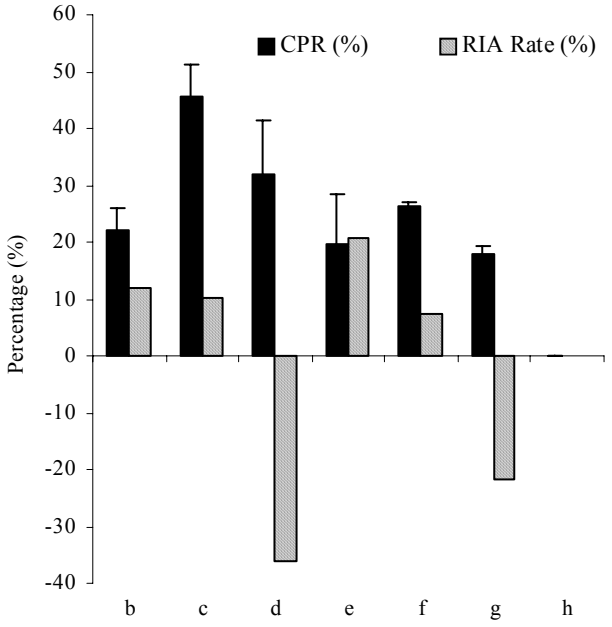

Figure 3. Effects of the MCE on cell proliferation rate and extracellular insulin concentration of alloxan damaged HIT-T15 cells

a.2% MCE, b. 0.2% MCE, c. 0.02% MCE, d. 2% MHMF, e. 2% MLMF, f. fresh juice, g. cold water extract of sun-dried bitter gourd, h. negative control: alloxan damaged cells. CPR: Cell Proliferation Rate; RIA Rate: the percentage of insulin secretion changes. *: $p<0.05$; **: $p<0.01$; n=4.

With the intention of investigating the possible therapeutical effects of bitter gourd preparations on the abnormal pancreas β- cells, the HIT-T15 cells were damaged by 0.1 mM radical initiator alloxan to simulate the pathological status of diabetes and used to evaluate the bitter gourd preparations' effects.

As shown in Fig.3, 0.02% MCE (w/v) achieved the highest cell proliferation rate of 45.6% ($p<0.01$), while 0.2%MCE, MHMF and fresh juice of bitter gourd increased proliferation rates by 22.1% ($p<0.05$), 32.1% ($p<0.05$) and 26.3% ($p<0.01$), respectively. The cold water extracts of sun-dried bitter gourd and the MLMF increased this figure but not significantly ($p>0.05$).

Table 2. Effects of the MCE on SOD activity (n=4) and Cell proliferation rates and insulin secretion amount of alloxan damaged HIT-T15 Cells

Group	SOD activity (NU/mL)	Cell Proliferation Rate (%)	Cell RIA Rate (%)
2% MCE	19.74	-100	-85.5
0.2% MCE	ND	22.1*	12
0.02% MCE	ND	45.6**	10.2
2% MHMF	5.32	32.1*	-36.1
2% MLMF	19.84	19.6	20.7
Fresh juice of bitter gourd pulps	12.16	26.3**	7.2
2% cold water extracts of sun-dried bitter gourd	7.37	17.9	-21.9

ND: not determined, *: $p<0.05$, **: $p<0.01$

The MLMF increased the insulin secretion of alloxan damaged HIT-T15 cells the most strongly, and contributed the highest number of 20.7% (shown in Fig 3), while the MHMF and the cold water extract of sun-dried bitter gourd decreased the insulin secretion of these abnormal cells by 36.1% and 21.9% respectively. Other preparations, the MCE and the fresh juice, only increased the insulin secretion slightly.

In general, the bitter gourd preparations can repair the damaged HIT-T15 cells and the MCE showed the highest repairing activity. The lack of correlation between the cell repairing abilities and insulin secretion promoting activities indicated these two functions of bitter gourds might caused by their different active ingredients. The reversed results of the effects of MHMF and MLMF on the insulin secretion indicated that MHMF might be responsible for MCE's cell repairing activity and act as a negative insulin secretion regulator while MLMF acted as the positive insulin secretion regulator.

As shown in Table 2, the samples varied in SOD activities. 2 % MLMF and 2 % MCE showed high SOD activities of 19.84 NU/mL and 19.74 NU/mL, respectively. 2% (w/v) MHMF showed a very limited SOD activity with value of 5.32 NU/mL.

In this study, MCE (0.2% ≥concentration≥0.02%) and its high molecular weight fraction showed the extent of their effect on repairing the abnormal β-cells, which were damaged by free radicals. The SOD activity was not detectable within MCE (0.02%), which induced the highest cell proliferation rate. On the contrary, 2%MCE and MLMF shown the highest SOD activities, 19.74 Nu/mL and 19.84 Nu/mL, but they failed in improving the proliferation rate of alloxan damaged cells. Neither the cell repairing activity nor the insulin secretion promoting activity of MCE was correlated with its SOD activity.

High concentration of MCE (2% or higher) killed the cells, which was very possibly caused by the extraordinarily high osmotic pressure of MCE molecules. The results

indicated that MCE might have a CC_{50} between 2% and 0.2% (w/v), and a relative low effective concentration less than 0.02% (w/v). The values of EC_{50} and CC_{50} will be measured further.

Compared to the fresh juice and extract of sun-dried bitter gourd, MCE showed higher activity on cell repair. It could be hypothesized that the components, which contributed to the cell repairing activity, were only produced during the boiling water extraction.

MHMF showed cell repairing activity, and MLMF showed the activity of promoting insulin secretion. Therefore, as the result of these activities in synergy, MCE repaired the abnormal cells and sustained their insulin secretion around the normal level. Interestingly, the MHMF showed inhibitory effects on insulin secretion of normal cells and free radical damaged cells. The stimulation of insulin secretion as shown in Table 2 may be through a complicated mechanism, which involved a delicate balance between MHMF and MLMF.

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