EFFECT OF *MOMORDICA CHARANTIA* LINN. ON HUMAN SKELETAL MUSCLE CELL

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ABSTRACT

This study aimed to investigate the effect of *Momordica charantia* Linn. (MC) fruit juice extract on glucose uptake level in human skeletal muscle cell. The MC juice was extracted using juicer without additional water to obtain pure juice. Then, glucose concentration level in MC was determined to indicate the existed glucose content in the juice using D-Glucose R-Biopharm assay kit. The same batch of juice was used to further study the effect of glucose uptake level in human skeletal muscle cell when treated with and without MC juice. The data then was analyzed using statistic. In brief, 42 mL of juice can be extracted from each 100g of MC fruit. The glucose concentration level in MC fruit extract was 6.8241g/L. The glucose concentration level in cells treated with DMEM only was 4.422 g/L and significantly higher ($p < 0.001$) than cells treated with DMEM added with MC juice, 3.678 g/L. This result indicated that the cells treated with MC juice had increased glucose uptake and utilization, leading to a reduction of glucose concentration level in media. This study demonstrated that the beneficial effect of MC juice extract on human skeletal muscle cell glucose uptake.

Keywords: *Momordica charantia* Linn.; glucose uptake; human skeletal muscle cell

1. INTRODUCTION

Momordica charantia Linn. (MC) or bitter gourd is known for its bitter taste comes from Cucurbitaceae family. This plant was used in Ayurveda therapeutic treatment as described in Ayurvedic texts written in Indian Sanskrit from 2000 to 200 BCE during Indo-Aryan culture [1] which indicates the early sign of MC cultivation in India [2]. In traditional medicinal practice, MC is used as anti-inflammatory, anti-ulcer, anti-leukemic, anti-HIV, anti-tumor, anti-diabetic, and anti-microbial [3], [4]. In diabetic studies, charantin, vicine and polypeptide-p extracted from fruit and seeds [5], [6] are the bioactive compounds in MC that have similar structure to insulin [7]. This similarities may contribute to MC abilities to increase insulin secretion, body tissue glucose uptake, glycogen synthesis in liver muscle and reduce glucose absorption [8]. These components and several type of extraction including pure juice, methanolic, ethanolic and chloroform extracts from the fruit, shows positive metabolic and hypoglycemic activity conducted on human, animal and cell culture research [9], [10].

Glucose uptake stimulation in insulin-sensitive tissues is one of crucial mechanism by which insulin controls blood glucose in human body [11]. In this study, human skeletal muscle cells were selected as it plays crucial role in energy homeostasis and insulin sensitivity. Skeletal muscle glucose uptake is the major site for insulin-regulated glucose clearance which mediated by GLUT-4 and GLUT-1 proteins, the major glucose transporter (GLUT) [12]. Skeletal muscle also responsible for the disposal of most of both oral and intravenous glucose loads for glucose

homeostasis [12], [13]. In type 2 diabetes mellitus (T2DM), the insulin resistance occurred due to the decreased of insulin-stimulated glucose transport and impaired metabolism in adipocytes and skeletal muscle. Eventually contributed to the down-regulation GLUT-4 which acts as the major-insulin responsive [14]. Although there are lots of studies that links MC extract effect to glucose uptake, to the current knowledge, there are still limited reports on glucose uptake activity exerted by MC extract in vitro studies. Thus, this study aims to investigate the relationship between glucose uptake and *Momordica charantia* Linn. fruit juice extract treated in in human skeletal muscle cell.

2. MATERIALS AND METHODS

2.1 Plant material and juice extraction

The unripe MC fruit were obtained from the local market, washed thoroughly and the seeds were removed. The fresh juice was prepared on a juicer without adding water to extract pure MC juice. The 100% juice was stored in 4°C until further use.

2.2 Determination of glucose level in MC juice extract

The glucose level in MC juice extract was determined by using D-Glucose R-Biopharm assay kit. For the solutions preparation, Bottle 1 (consisting of triethanolamine buffer, pH 7.6; NADP, 110 mg; ATP, 260 mg; magnesium sulphate) was dissolved in 45 ml redistilled water and the Bottle 2 (consisting of 1.1 mL suspension containing hexokinase, 320 U; glucose-6-phosphate dehydrogenase, 160 U) was used undiluted. As for controls, D-glucose assay solution (Bottle 3) was used. Then, the solutions were pipetted into cuvettes as in Table 1. The absorbance reading was taken with Shimadzu UV-1800 spectrophotometer and the glucose concentration was calculated.

Table 1: Assay kit solutions preparation

Pipette into cuvettes	Blank (mL)	Sample (mL)
Solution 1	0.500	0.500
Sample solution*	-	0.050
Redistilled water	1.000	0.850
The solution* was mixed and the absorbance was read (A_1) approximately after 3 minutes. The reaction then was started by adding:		
Suspension 2	0.010	0.010
The solution* was mixed and waited for the action to stop approximately in 10-15 minutes and the absorbance of the solutions was read (A_2). However, if the reaction does not come to stop after 15 min, the absorbance read will be continued at 2 min intervals until the absorbance increases constantly for 2 min.		

2.3 Determination of MC juice extract on human skeletal muscle cell glucose level

Human skeletal muscle cells was cultured in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% of fetal bovine serum (FBS) and 1% of penicillin-streptomycin. The cells were sub-cultured every three days until the cells reached 70%-80% of confluent culture. 2-4 x

10^4cells/cm^2 were used as standard seeding density and then cultured in 75cm^2 flask. The cell culture was incubated in a humidified atmosphere that contained 5% CO^2 at 37°C and further maintained as stated before. For the differentiation induction, the cells were seeded at a density of 2-8 x 10^4cells/ml in 96-well plate and supplemented with Skeletal Muscle Cell Differentiation Medium (SKM-D). 100µL of cell solution were pipetted into each well. The cells were maintained until reaching the 70%-80% of confluent culture for about four to six days. Supporting growth medium was changed every other day and cells were washed with phosphate buffer saline (PBS). The cells confluence was monitored using an inverted microscope. To study the effect of MC juice extract on human skeletal muscle cell, the cells were treated with and without MC juice extract in 96 well-plate. The cells were discarded and the media was collected and further analyzed according to D-Glucose R-Biopharm glucose uptake assay protocol.

2.4 Statistical analysis

The obtained data were collected and analyzed using standard t-test where $p<0.05$ is considered significant. The data were presented in bar graph.

3. RESULTS AND DISCUSSION

3.1 Juice concentration

Concentration of the juice was expressed in relation to wet weight which is 42 mL obtained from 100g of MC fruits.

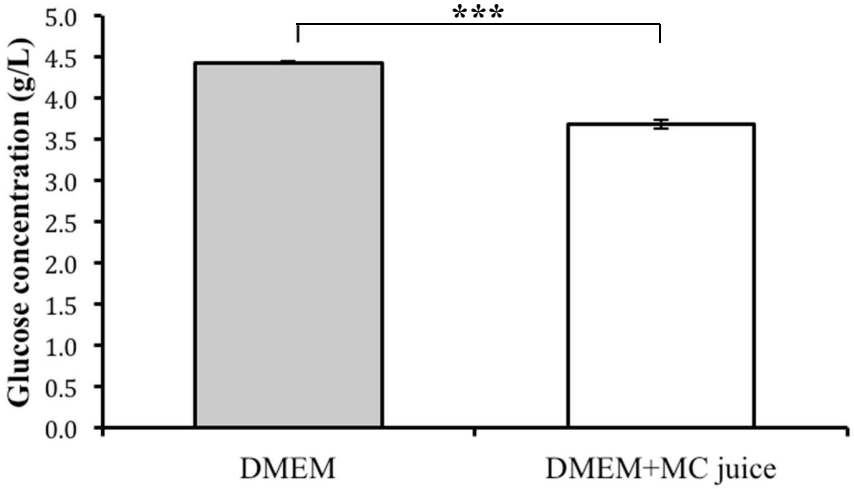
3.2 Glucose concentration level in MC juice extract

The glucose concentration of pure MC juice extract was determined using D-Glucose R-Biopharm assay kit with three replicates. From the experiment, we found out that the glucose concentration level in MC juice extract was 6.8241g/L.

3.3 Effect of MC juice extract on human skeletal muscle cell glucose level

In this study, an experiment was conducted on two different set human skeletal muscle cell treatments with three replications each. In Sample 1, the cells were treated with only DMEM media. Meanwhile in Sample 2, the cells were treated with DMEM added with MC juice. As presented in Graph 1, we found out that the glucose concentration level in cells treated with DMEM only was 4.422 g/L and significantly higher ($p<0.001$) than cells treated with DMEM added with MC juice, 3.678 g/L. This indicated that the cells treated with MC juice worked actively in glucose utilization that leads the cells to improve glucose uptake thus leads to the reduction of glucose concentration level in media in Sample 2. According to study by Lee et. al, they proposed two possible mechanism of MC extracts on blood; (1) MC extract has the abilities to regulate glucose content absorbed to the gut into the blood following a meal and; (2) the MC extracts has hypoglycemic effect and insulin-like properties which can stimulate the glucose uptake into skeletal muscle cell [15]. Isolated bioactive compound such as charantin, vicine and polypeptide-p extracted from fruit and seeds [5], [6] are responsible to exert potential effects in lowering blood glucose by increasing the glucose uptake and glycogen synthesis in the liver, muscles, and adipose tissue and activating insulin receptor substrate 1 (IRS1) in skeletal muscle by tyrosine phosphorylation [16]. There are

also studies that reports the hypoglycemic effects of MC extracts were occurred due to the increase of beta cells number, glucose uptake by skeletal muscle and adipose tissue and AMPK pathway activation that contributes to insulin secretion stimulations [17].



Graph 1: Glucose concentration level in human skeletal muscle treated with and without MC fruit juice. *** $p < 0.001$

4. CONCLUSION

From this study, it can be concluded that *Momordica charantia* Linn. fruit juice extract has shown anti-diabetic properties, which helped in increase glucose uptake into the cell thus reducing glucose level in blood glucose. For further studies, it is recommended to explore the optimum MC concentration intake for optimized the glucose uptake intake. Also, studying the effect of MC different varieties and its bioactive compound will help us to explore more on the potential of MC anti-diabetic activities. In addition, the ongoing work of this study includes the investigation of anti-inflammatory effect of *Momordica charantia* Linn.

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