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Momordica charantia fruit juice stimulates glucose and amino acid uptakes in L6 myotubes

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Abstract

The fruit of *Momordica charantia* (family: Cucurbitacea) is used widely as a hypoglycaemic agent to treat diabetes mellitus (DM). The mechanism of the hypoglycaemic action of *M. charantia in vitro* is not fully understood. This study investigated the effect of *M. charantia* juice on either ^{3}H-2-deoxyglucose or N-methyl-amino-*a*-isobutyric acid (^{14}C-Me-AIB) uptake in L6 rat muscle cells cultured to the myotube stage. The fresh juice was centrifuged at 5000 rpm and the supernatant lyophilised. L6 myotubes were incubated with either insulin (100 nM), different concentrations (1–10 μg ml^{-1}) of the juice or its chloroform extract or wortmannin (100 nM) over a period of 1– 6 h. The results were expressed as pmol min^{-1} (mg cell protein)$^{-1}$, n = 6–8 for each value. Basal ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes by L6 myotubes after 1 h of incubation were (means $\pm$ S.E.M.) 32.14 $\pm$ 1.34 and 13.48 $\pm$ 1.86 pmol min^{-1} (mg cell protein)$^{-1}$, respectively. Incubation of L6 myotubes with 100 nM insulin for 1 h resulted in significant (ANOVA, $p < 0.05$) increases in ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes. Typically, ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes in the presence of insulin were 58.57 $\pm$ 4.49 and 29.52 $\pm$ 3.41 pmol min^{-1} (mg cell protein^{-1}), respectively. Incubation of L6 myotubes with three different concentrations (1, 5 and 10 μg ml^{-1}) of either the lyophilised juice or its chloroform extract resulted in time-dependent increases in ^{3}H-deoxy-D-glucose and ^{14}C-Me-AIB uptakes, with maximal uptakes occurring at a concentration of 5 μg ml^{-1}. Incubation of either insulin or the juice in the presence of wortmannin (a phosphatidylinositol 3-kinase inhibitor) resulted in a marked inhibition of ^{3}H-deoxyglucose by L6 myotubes compared to the uptake obtained with either insulin or the juice alone. The results indicate that *M. charantia* fruit juice acts like insulin to exert its hypoglycaemic effect and moreover, it can stimulate amino acid uptake into skeletal muscle cells just like insulin. (Mol Cell Biochem **261**: 99–104, 2004)

Key words: L6 myotubes, insulin, *Momordica charantia*, glucose, amino acid, wortmannin

Introduction

Diabetes mellitus (DM) is the most common of the endocrine disorders and a major global health problem today [1, 2]. DM is characterised by chronic hyperglycaemia as a result of a relative or absolute lack of insulin, or the actions of insulin on its target tissues or both [3]. Insulin is the mainstay for patients with type 1 diabetes and it is also important in type 2 when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications [3]. Previous to the use of insulin, dietary measures were the major form of this treatment especially in many developing countries. This includes the traditional medicines derived from plants. A multitude of plants have been used for the treatment of diabetes throughout the world [4]. One such plant is *Momordica (M) charantia* Linn (Family: Cucurbitacea), whose fruit is known as *Karella* or bitter gourd [5–9]. For a long time, several workers have studied the hypoglycaemic effects of this

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plant in diabetes [5]. The treatment with *M. charantia* fruit juice reduced blood glucose levels, improved body weight and glucose tolerance [8–14]. Another action of the juice of this plant is to preserve and regenerate damaged pancreatic islet beta cells and beta cells functions resulting in a significant increase in insulin secretory activity [8, 12, 13].

In addition, previous studies have shown both the aqueous and alcoholic extracts of the fruit possess hypoglycaemic activity in streptozotocin-induced diabetic rats by inhibiting the enzyme fructose 1, 6-diphosphatase and glucose 6 phosphatase and at the same time stimulating the enzyme glucose 6 phosphate dehydrogenase [5, 7, 9]. However, the cellular mechanism of action of the hypoglycaemic activity of *M. charantia* is not fully understood. Some studies have suggested that *M. charantia* juice and its extract can stimulate peripheral glucose uptake [5, 7] and moreover, regulate the amount of glucose taken up by the gut [5, 9]. In light of these suggestions, this study was designed to investigate the effect of *M. charantia* fruit juice extracts on ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes in L6 rat muscle cells. The effects of insulin and wortmannin were also investigated for comparison.

Materials and method

Preparation of the juice and its extract

Fresh *M. charantia* unripe fruits were obtained from the local market and cut to remove the seeds. The fruits were chopped into small pieces and then homogenised with a commercial blender. The fresh juice was then centrifuged at 5000 rpm and the supernatant lyophilised (Fraction A). Fraction B was extracted from Fraction A using chloroform and subsequently lyophilised [5, 9]. Both extracts A and B were employed to determine the effect of *M. charantia* on glucose and amino acid uptakes in L5 rat muscle cells since it is the perception that the A and B extracts contained the hypoglycaemic active ingredients.

L6 muscle cell culture

Monolayers of L6 muscle cell culture were prepared by a previously established method [15–17]. The cells were grown to the differentiated stage of myotubes in a minimal essential medium (MEM), containing 5 mM glucose in the presence of 2% fetal bovine serum and 1% antimycotic solution and penicillin (100 μg ml^{-1} penicillin G, 100 μg ml^{-1} streptomycin and 250 mg ml^{-1} amphotercin B), in an atmosphere of 5% carbon dioxide and 5% oxygen at 37°C. The differentiated myotubes were then used to investigate the effects of *M. charantia* juice and its extract on ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes.

*Assay for radiolabelled ^{3}H-deoxy-*D*-glucose and amino acid transport*

Cultures were prepared according to well-established methods [15, 17]. Confluent cultures at the stage of myotubes were incubated in serum free media overnight with 25 mM glucose. Next morning the cells were washed with Hanks Balanced Salt solution (HBS) containing 5 mM glucose. The cells were then incubated in this HBS solution containing either 100 nM insulin alone or different concentrations of either A or B extract (1 μg–10 μg) at 37°C. A few experiments were also done with either 100 nM wortmannin alone or wortmannin in combination with either 100 nM insulin or 10 μg ml of extract A or B. The cells were incubated with the different stimulants for either 1, 3 or 6 h and control experiments in the absence of any stimulant were also performed for the same incubation periods.

At the end of the incubation, the cells were rinsed with glucose free HBS and the uptake of either glucose or amino acid (a paradigm system) was quantified using 10 μM of ^{3}H-deoxyglucose (μCi ml^{-1}) or 10 μM ^{14}C-Me-AIB (10 μCi ml^{-1}). Non-specific glucose uptake was determined in the presence of 10 μM cytochalasin-B and was subtracted from the total uptake. For the amino acid this was determined in the presence of 1 mM unlabelled Me-AIB. Both uptakes were performed simultaneously for 10 min followed by rapid aspiration of the radioactive medium and three successive washes with icecold normal saline for 5 min each. The cells were then lysed in 50 mM NaOH followed by liquid scintillation counting. Total cell protein was then determined using the method of Bradford [18] and both glucose and amino acid uptakes were expressed in p mol min^{-1} (mg cell protein)$^{-1}$.

Statistical analysis

All data are expressed as means ± standard error of the mean (S.E.M.). The significance between independent variables was performed by a priori test ANOVA and a positiori Newman Keuls test. The level of $p < 0.05$ was considered significant.

Results

Figure 1 shows the time-course effects (1, 3 and 6 h) of incubating L6 myotubes with either 100 nM insulin or 5 ug ml^{-1} of either extract A or B on ^{3}H-deoxyglucose uptake. The control uptake is also shown in the figure for comparison. The results show that either insulin or extract A or B can evoke significant ($p < 0.05$) increases in glucose uptake following 6 h of incubation compared to control uptake. Incubation of L6 myotubes for 1 and 3 h resulted in small increases in

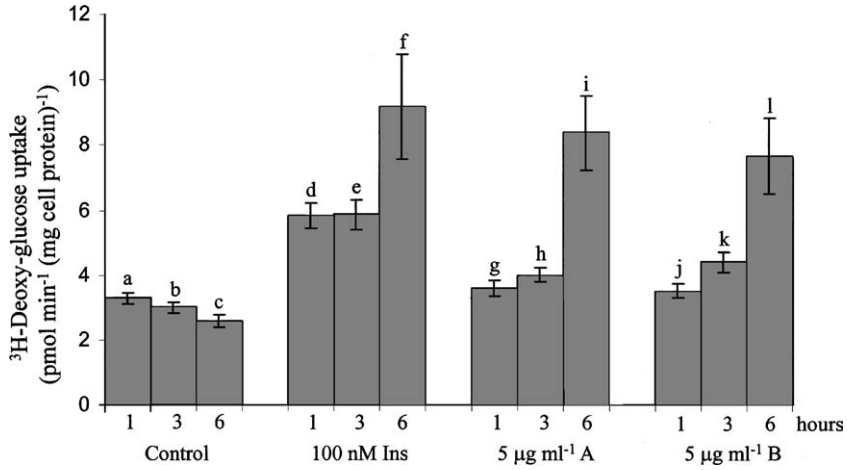

Fig. 1. Time-course effect of incubating L6 myotubes with either 100 nM insulin (Ins, d–f), 5 μg ml^{-1} extract A (g–i) or 5 ug ml^{-1} extract B (j–l) for 1, 3 and 6 h in the presence of ^{3}H-deoxyglucose. The control uptake for 1, 3 and 6 h (a–c) are also shown in the figure for comparison. The results show significant increases in ^{3}H-deoxyglucose uptake for either insulin (Ins), extract A or B compared to their respective time-course controls. For c, $p < 0.001$ compared to f, i and l. Each point is mean ± S.E.M., $n = 10$.

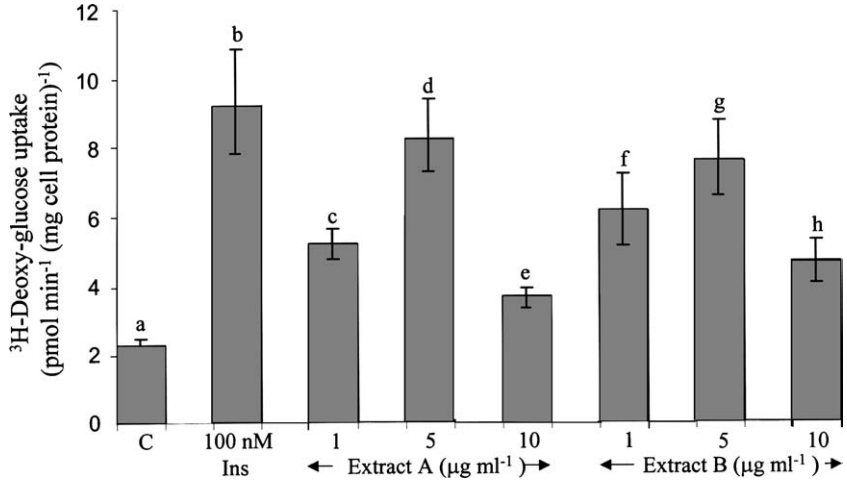

Fig. 2. Bar charts showing ^{3}H-deoxyglucose uptake by L6 myotubes in control condition (a), in the presence of 100 nM insulin (b) and in the presence of different concentrations (1–10 μg ml^{-1}) of either extract A (c, d and e) or B (f, g and h) over a period of 6 h. The results shown significant ($p < 0.01$) increases in glucose uptake for either insulin (Ins), extract A or B compared to control. Note that lower concentrations of the extracts were more effective in stimulating glucose uptake. Each point is mean ± S.E.M., $n = 10$.

^{3}H-deoxyglucose uptake compared to the responses obtained after 6 h of incubation. Figure 2 shows the effect of different concentrations (1, 5 and 10 ug ml^{-1}) of either extract A or B on ^{3}H-deoxyglucose uptake in L6 myotubes following 6 h of incubation. The effect of 100 nM insulin and the control uptake are also shown for comparison. The results reveal that lower concentrations of either extract A or B are more effective in stimulating glucose uptake compared to higher concentration. The effects of 1 μg ml^{-1} and 5 μg ml^{-1} were much larger compared to the response obtained with 10 μg ml^{-1}.

Since both insulin and extracts A and B can stimulate ^{3}H-deoxyglucose uptake, it was decided to determine the signal transduction pathways associated with the uptake. In this series of experiments, the phosphatidylinositol 3-kinase inhibitor, wortmannin was employed either alone or in combination with either 100 nM insulin or 10 μg ml^{-1} either extract A or B. The results are shown in Fig. 3. The results clearly show that either insulin, extract A or B, can evoke significant ($p < 0.01$) increases in ^{3}H-deoxyglucose uptake in L6 myotubes compared to control. Incubation of L6 muscle cells with 100 nM wortmannin alone resulted in a significant ($p < 0.05$) decrease in ^{3}H-deoxyglucose uptake compared to control. Combining wortmannin with either insulin or extract A or B resulted in significant ($p < 0.001$) decreases in

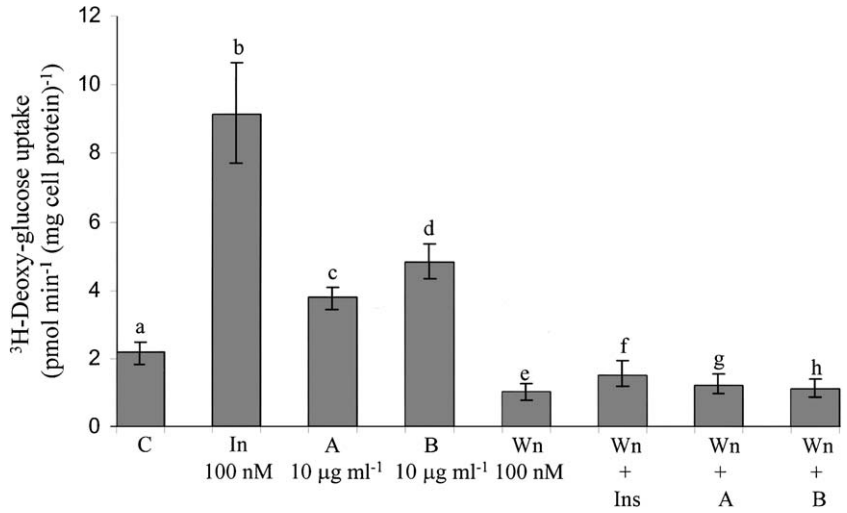

Fig. 3. Bar charts showing ^{3}H-deoxyglucose uptake in L6 myotubes for 6 h in controlled condition (a) and following incubation with either 100 nM insulin (Ins; b), 10 μg ml^{-1} of extract A (c) or B (d) in the absence and presence of 100 nM wortmannin (Wn) (compare b with f, c with g and d with h). The effect of Wn alone is also shown for comparison (e). The results show that Wn can significantly ($p < 0.05$) inhibit the uptake of ^{3}H-deoxyglucose in control condition and in response to either Ins, extract A or B. Each point is mean ± S.E.M., $n = 10$.

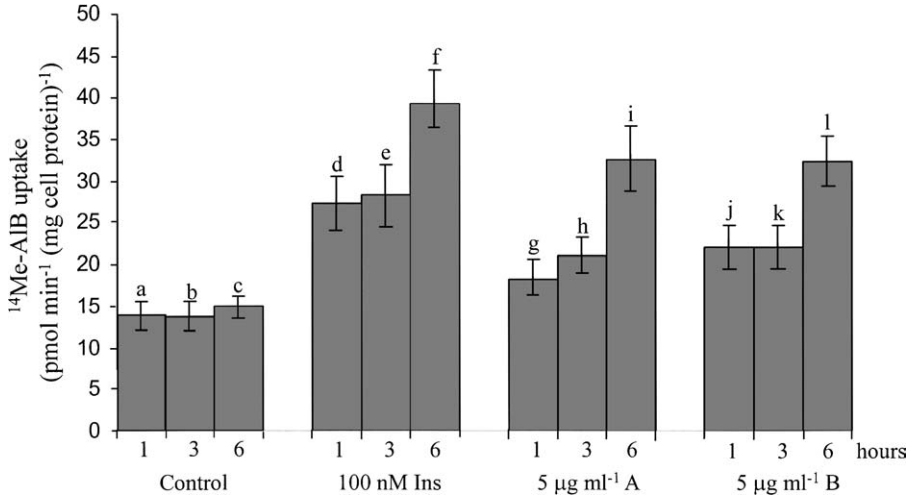

Fig. 4. Time-course uptake (1, 3 and 6 h) of ^{14}C-Me-AIB by L6 myotubes in control state (a–c) and following incubation with either 100 nM insulin (Ins, d–f), or 5 ug ml^{-1} of either extract A (g–i) or B (j–l). Data are mean ± S.E.M., ($n = 10$). Note that both insulin and extracts A and B can evoke significant ($p < 0.001$) increases in ^{14}C-Me-AIB uptake compared to the respective controls.

^{3}H-deoxyglucose uptake compared to the responses obtained with either insulin, extract A or B alone. These results indicate that both extracts A and B are exerting their effects on ^{3}H-deoxyglucose uptake in L6 myotubes similar to that of insulin since wortmannin can exert the same inhibiting effects on all three agents.

Since both extracts A and B can stimulate ^{3}H-deoxyglucose uptake in L6 myotubes, it was decided to investigate the effects of these extracts on ^{14}C-Me-AIB uptake. The effect of insulin was also investigated for comparison. Figure 4 shows the time-course effect (1, 3 and 6 h) of either 100 nM insulin or 5 μg ml^{-1} of either extract A or B on ^{14}C-Me-AIB uptake in L6 myotubes. The control uptake is also shown in the figure for comparison. The results show that either insulin, extract A or B can all stimulate ^{14}C-Me-AIB uptake with maximal uptake occurring after 6 h of incubation. The effect of 5 μg ml of either extract A or B was similar to that of 100 nM insulin. Figure 5 shows the effect of different concentrations (1, 5 and 10 μg ml^{-1}) of either extracts A or B on ^{14}C-Me-AIB uptake over a duration of 6 h. The effect of 100 nM insulin and the control uptake are also shown for comparison in Fig. 5. Like ^{3}H-deoxy-D-glucose uptake (see Fig. 2), the results here show that both 1 μg ml^{-1} and 5 μg ml^{-1} of either extract A or B can stimulate ^{14}C-Me-AIB

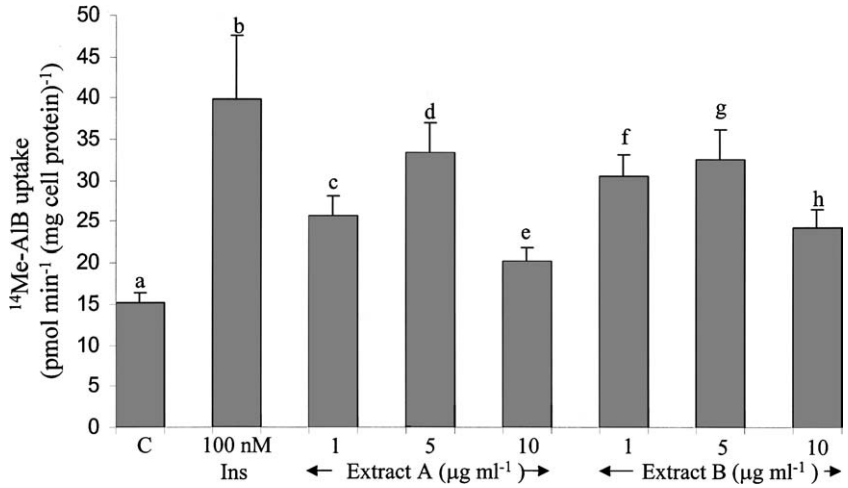

Fig. 5. Bar chars showing ^{14}C-Me-AIB uptake by L6 myotubes over a duration of 6 h following incubation with either 100 nM insulin (Ins; b) or 1–10 μg ml^{-1} of either extract A (c–e) or B (f–h). The control uptake (a) is also shown for comparison. Each point is mean ± S.E.M., $n = 10$. Note that either insulin, extract A or B can elict significant ($p < 0.01$) increases in ^{14}C-Me-AIB uptake in L6 myotubes compared to control.

uptake in a dose-dependent manner. In contrast, 10 μg ml^{-1} evoke a much smaller effect compared to the two concentrations. The effect of 5 μg ml^{-1} was more or less similar to the effect of 100 nM insulin.

Discussion

The results of this study have clearly demonstrated that the metabolic hormone insulin, at a physiological concentration, can elicit significant ($p < 0.05$) time-dependent increases in ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes in L6 muscle cells compared to the respective controls. Similarly, incubation of L6 muscle cells with either the lyophilised extract or the chloroform extract of *Mormordica charantia* can result in marked time-dependent increases in ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes. The magnitude of the uptakes was dependent on both the duration of the incubation and the concentrations of the extracts, similar to the effects observed with insulin. Incubation for either 1 or 3 h had small, but clear increases in the uptakes of ^{3}H-deoxyglucose and ^{14}C-Me-AIB by L6 muscle cells. In contrast, at 6 h of incubation, there were significant ($p < 0.01$) increases in uptake compared to the respective controls. The results have also shown that lower concentrations (1 μg ml^{-1} and 5 μg ml^{-1}) of the extracts were more potent in stimulating both ^{3}H-deoxyglucose and ^{14}C-Me-AIB uptakes compared to a higher concentration of 10 μg ml^{-1}. In a previous study, it was also shown that both extracts A and B at concentrations of 50–200 μg ml^{-1} not only inhibited at ^{3}H-deoxyglucose uptake alone, but also attenuated the stimulating response to insulin [9, 13]. In fact in the present study, it has been shown that the effect of 5 μg ml^{-1} of either extract was more or less the same as 100 nM insulin.

It is now well established that insulin can stimulate glucose uptake into muscle cells employing the phosphatidylinositol 3-kinase pathway since this enzyme can be inhibited by wortmannin [15–17]. However, the mechanism of action of *M. charantia* extracts on glucose uptake into L6 myotubes is still unclear. The results of this study have shown that when wortmannin was combined with either extract A or B, there was a marked and significant ($p < 0.01$) attenuation of ^{3}H-deoxyglucose uptake by L6 myotubes similar to the response obtained from insulin in combination with wortmannin. Taken together, these results have demonstrated that the active ingredient(s) of *M. charantia* is exerting its effect just like insulin to bring about a hypoglycaemic effect. In addition, the results have also demonstrated that the concentration of the extract is very important in stimulating glucose uptake into muscle cells. Pharmacological concentrations have inhibitory effect [9, 13] whereas physiological concentrations have insulin-like stimulating effects. In a few previous studies (see Platel and Srinivasan [5]), it was shown that *M. charantia* had no beneficial effect in regulating blood glucose level *in vivo*, whereas in several other studies *M. charantia* and its extracts had marked beneficial effects. In reviewing the literature [5], it could be seen that all the studies, which reported beneficial effects employed low concentrations of *M. charantia* and its extracts whereas the studies that reported non- beneficial effects employed very high pharmacological doses of *M. charantia* and its extracts.

The results of this study have also demonstrated that L6 muscle cells can take up ^{14}C-ME-AIB in response to either insulin or extract A or B. There results are in agreement with

previous studies [15, 17, 19] in which L6 myotubes can take ^{14}C-Me-AIB in a time-dependent manner and the magnitude of the uptake was dependent upon the concentration of insulin. The effect of 5 μg ml^{-1} of either extract A or B was similar to that of 100 nM insulin on ^{14}C-Me-AIB uptake. Taken together, these results further strengthen an insulin-like activity of *M. charantia* fruit extracts. In a previous study [20], it was demonstrated that the juice of *M. charantia* contains an insulin-like peptide that lowers blood glucose level. Moreover, the peptide can also stimulate insulin-dependent enzymes such as hexokinase which is responsible for protein anabolism. The beneficial effects of *M. charantia* on amino acid uptake may be related to protein synthesis in muscle cells and makes possible the synthesis of glucose transporters.

In conclusion, the results have clearly demonstrated that fruit juice extracts of *M. charantia* can stimulate glucose and amino acid uptakes into L6 muscle cells just like insulin. Taken together, the results support the beneficial use of *M. charantia* in the treatment of diabetes mellitus.

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