Antidiabetic Activity of *Momordica charantia* L. and Mechanism of Insulin Secretion of 1-Butanol Soluble Part on Type 2 Diabetic Model Rats

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ABSTRACT: The *in vivo* effects of fruit pulp juice (MC-PJ) of *Momordica charantia* and its 1-butanol soluble part (MC-BP) and aqueous soluble part (MC-AP) on blood glucose of type 2 diabetic rats were studied. *In vitro* insulin secretion in response to MC-BP and MC-AP from whole perfused pancreas was measured. For elucidating the mechanism of insulinotropic action, the insulin secretory activity of MC-BP in the presence of 11 mM glucose, 50 μM verapamil (Ca^{++} channel blocker), 8 mM diazoxide (K^{+}_{ATP} channel opener) and 10 mM theophylline (cAMP phosphodiesterase inhibitor) were studied. Serum glucose was measured by glucose oxidase-peroxidase method and rat insulin was assayed by specific ELISA. In the *in vivo* study, MC-BP significantly opposed the rise of serum glucose compared to control at 105 min ($p<0.05$). Although the MC-AP and MC-PJ lowered the serum glucose both at 60 and 105 min, these were not statistically significant. In the *in vitro* study, only MC-BP produced 22-fold increase in insulin secretion from the perfused pancreas at nonstimulatory glucose level, which was significant (basal vs. MC-BP, 0.071±0.009 vs. 1.563±0.150 ng/ml, $p<0.001$). The MC-BP also enhanced the insulin secretion from the glucose-stimulated pancreas ($p<0.001$). The MC-BP induced insulin secretion was not affected in presence of diazoxide and verapamil. The obtained results also showed that MC-BP enhanced the insulin secretory effect of theophylline ($p<0.001$). The findings indicate that MC-BP has stimulatory effects on physiological pathways of insulin secretion which may underlie its reported antidiabetic action.

Key words: *Momordica charantia*, antidiabetic effect, insulin secretion, pancreatic perfusion

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism characterized by high blood glucose levels (hyperglycemia) and usually resulting from insufficient production of the hormone insulin (type 1 diabetes) or an ineffective response of cells to insulin (type 2 diabetes). It is considered as a fastest growing disease in the world.[1] Many studies have suggested that different extracts of *M. charantia* can be used as traditional remedy for the treatment of diabetes.[2-9]

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Crude extracts from its fruits showed hypoglycemic activities in animals and clinical trials has previously been reported.[10-14] The aqueous extract of *M. charantia* (2.5 g/kg body weight) is known to produce 45% hypoglycemic activity in normal rats after four hours of treatment.[15] On the other hand, 34% hypoglycemic activity of water soluble fraction of *M. charantia* was detected after the third hour in streptozotocin induced diabetic rats.[16] The antihyperglycemic activity of *M. charantia* fruit juice was found when administered orally to human adult (100 ml juice 30 min before the oral glucose load), which improved glucose tolerance in 73% of the patients investigated.[7] Oral administration of the

aqueous extract of *M. charantia* fruits significantly lowered blood glucose level in streptozotocin (STZ)-induced diabetic rats at a dose of 250 mg/kg body weight.[17] A double blind placebo controlled trial with *M. charantia* was performed, which concluded that the effect of *M. charantia* capsule preparation on glycemic control in type 2 diabetes mellitus needs further studies.[18] It was shown that *M. charantia* extracts possesses anti-diabetic, hepato-renal protective and hypolipidemic effects in alloxan-induced diabetic rats.[19] In addition, juice made with *M. charantia* significantly reduced cellular TG and microsomal TG transfer protein, suggesting that lipid bioavailability and lipidation of apolipoprotein B assembly may involve in decreased apolipoprotein B secretion.[20] Another study showed that the aqueous extract of *M. charantia* fruit has hypoglycaemic activity in cyproheptadine-induced diabetic mice.[21] When administered orally *M. charantia* aqueous extracts lowered intestinal glucose absorption and involved extra-pancreatic effects.[12] The available literature shows that D-chiro-inositol found in *M. charantia* fruit extract plays a role in reducing blood glucose in streptozotocin diabetic rats.[22] Blood glucose tolerance in alloxan-induced rats was ameliorated significantly from day 7 to day 22 and reduced to normal levels with the chronic administration of the *M. charantia* fruit juice orally at a dose of 20 mg/kg body weight.[23] The mechanism of antidiabetic action by *M. charantia* extracts may be due to enhancing insulin secretion by the islets of Langerhans, enhancing peripheral glucose utilization, increasing serum protein levels and reducing glycogenesis in liver tissue.[24] Our previous work on fruit pulp, seed and whole plant of *M. charantia* on normal and diabetic rats showed that pulp juice lowered fasting blood glucose levels in normal rats at 120 min and the effect was more pronounced with the saponins-free methanol extract of the pulp juice at 60 min and 120 min.[25] The pulp juice also had a significant hypoglycemic effect in the glucose-fed normal rats when the extract was fed 45 min before the oral glucose load.[25] The saponins-free methanol extract of juice produced a significant hypoglycemic effect both in fasting and postprandial states in type 2 model rats.[25] Later a follow-up *in vitro* study with two fractions from saponins-free methanol extracts of *M. charantia* dried fruit pulp showed significant insulin-releasing activity at 3 mM glucose concentration.[26] It was also shown that these two fractions also stimulated influx of Ca^{2+} in the β cells.[27] The present study reports the *in vivo* effects of, 1-butanol soluble part and aqueous part of *M. charantia* fruit pulp juice on type 2 diabetic rats and *in vitro* effects on insulin secretion from whole perfused pancreas for elucidating the mechanism of insulinotropic action.

MATERIALS AND METHODS

Plant materials. Fresh fruits of *Momordica charantia* L. were bought from a local market of Dhaka city. The fruits were washed with clean water and the adhering water was removed by air-drying. Each of the fruit was divided into two halves with a sharp knife and the seeds were removed using a teaspoon.

Preparation of juice of *M. charantia* fruit. The fresh fruit pulp was crushed in a kitchen blender (Philips, China) and the juice was squeezed out with pre-cleaned cloth filter. The residue was further blended with an ultra-turrax adding some fresh juice to the pulp and more juice was squeezed with cloth filter. All the juice was centrifuged and the clear juice was immediately stored in a deep freezer (-20°C).

Liquid-liquid extraction of pure juice. The centrifuged clear juice was partitioned with 1-butanol using a separating funnel (1000 mL). Both 1-butanol soluble part and aqueous part was separately collected, concentrated and freeze-dried. Finally, 1-butanol soluble part, aqueous part and centrifuged freeze-dried juice were selected for biological testing.

Animals. Male Long-Evans rats (180-220 g) breed at BIRDEM animal house were used in this study. Type 2 diabetes was induced by injecting streptozotocin intraperitoneally to 48h old pups according to Bonner-Weir.[28] Three months later the rats were checked with an OGTT and taken for the experiment.

***In vivo* experiment.** The *in vivo* effect of the extractives (direct juice, 1-butanol soluble part and aqueous part) were studied in rate model. The extractives were administered (1.25g/kg body weight) to the rats 30 min before the oral glucose load at a dose of 250 mg/kg body weight. Blood samples were collected by cutting the tail tip under mild ether anesthesia at different time points (Table 1).

Isolated pancreas perfusion technique. *In vitro* insulin secretory effect was investigated with 3-month-old rats using the isolated perfused pancreas technique.[29] Long-Evans rats (220-250 g) were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and pancreas was isolated with the proximal portion of the duodenum and separated from the spleen and stomach. The pancreas plus duodenum block was perfused through the celiac and mesenteric arteries via a cannula inserted into the aorta at a constant (37°C) temperature without recycling the medium. The perfusate was a Krebs-Ringer bicarbonate buffer (KRBB) with the following components: 2.8 mmol/l D-glucose (Merck, Germany), 118 mmol/l NaCl, 4 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 25 mmol/l $NaHCO_3$, 1.25 g/l fatty acid-free BSA (Sigma) and 40 g/l dextran T70 (Pharmacia, Sweden). The perfusate was continuously gassed with a mixture of O_2:CO_2 (95 : 5). Pancreas was first perfused with KRBB during a 20-min equilibration period after the surgery. The effluent of the final 10 min was collected at 1-min intervals for determination of the basal rate of insulin release. The stimulatory effects of glucose and arginine were then investigated sequentially. After a 10-min basal perfusion, the pancreas was perfused for 20 min with KRBB containing 11 mmol/l of D-glucose (Merck), followed by KRBB supplemented with 19 mmol/l L-arginine. The arginine perfusion was preceded and followed by a 10-min period of perfusion with KRBB containing 2.8 mmol/l D-glucose. The insulin secretory activity of 1-butanol soluble part of *M. charantia* was studied in presence of 11 mM glucose, 50 μM verapramil (Ca^{++} channel blocker), 8 mM diazoxide (a K^+ sensitive ATP channel opener) and 10 mM theophylline (a cAMP phosphodiesterase inhibitor). The complete effluent (1 ml/min) was collected from the cannula in the portal vein at 1-min intervals in chilled tubes and frozen for storage at -20°C until assay.

Analytical techniques. Glucose was measured by glucose-oxidase method.[30] Rat insulin (Crystal Chem, USA) was assayed by specific ELISA.[31]

Statistical analysis. The results are expressed as mean ± SD for a given number of observations (n). The significance of differences between mean values was evaluated by one-way ANOVA followed by a Scheffe's test. A 'p' value less than 0.05 was taken as significant.

RESULTS AND DISCUSSION

The results of *in vivo* study of oral administration of pure juice and sub-fractions of *M. charantia* 30 min before glucose load are presented in Table 1. As it is seen from the Table 1 oral administration of 1-butanol soluble part significantly opposed the rise of serum glucose compared to control at 105 min (Serum Glucose, mmol/l, M±SD: 14.9±2.6 vs. 17.5±1.1; $p < 0.05$). Aqueous soluble part and the pulp juice lowered the serum glucose both at 60 and 105 min.

The results of *in vitro* studies indicated that baseline values from control and *M. charantia* sub-fractions treated rats overlapped. As expected, in control perfusions, insulin output did not vary significantly throughout the experimental period (90 min, data not shown). In control experiments, 19 mM arginine was administered at the end of experiment to stimulate insulin secretion in order to confirm that the tissue retained the normal secretory capacity during 90 min of experiment.

This revealed a 3-fold increase in insulin release in response to 19 mM arginine compared to 11 mM glucose. The 1-butanol soluble part of *M. charantia* caused a significant ($p<0.001$) increase in insulin release during the 10 min perfusion, with a peak of 22 fold of the basal level of 0.071± 0.009 ng/mL to peak value of 1.563±0.150 ng/mL ($p<0.001$, Figure 1). As it is seen from Figure 1 that the aqueous

soluble part (MC-AP) did not stimulate insulin release significantly. Subsequent exposure for 5 min to 11 mM glucose caused a steep elevation in insulin release. When extract was perfused with 11 mM glucose it stimulated further insulin release, with a peak of 109% of 11 mM glucose (Table 2). During 10 min washout period following perfusion with MC-BP, the effluent insulin concentration remained high, although they were expected to return to the baseline level. Then the washout period was prolonged to 20 min and it returned to the baseline level indicating a high binding affinity of the extract.

Table 1. Effects of pulp juice, aqueous part and 1-butanol soluble part of *M. charantia* on serum glucose levels of type 2 diabetic rats when the extract was fed 30 minutes before glucose load.

Group	Min 0	Min 60	Min 105
WC (n=10)	7.7 ± 1.9	18.5 ± 3.5	17.5 ± 1.1
GB (n=10)	7.9 ± 1.0	16.5 ± 2.7	14.4 ± 1.8
MC-PJ (n=8)	7.3 ± 2.1	14.2 ± 3.1	16.6 ± 1.9
MC-AP (n=8)	7.4 ± 1.6	15.0 ± 3.0	15.5 ± 3.6
MC-BP (n=8)	8.4 ± 1.6	16.2 ± 2.5	14.9 ± 2.6
Bonferroni p value			
WC vs Gb	0.845	0.117	0.009
WC vs MC-PJ	0.756	0.093	0.143
WC vs MC-AP	0.738	0.103	0.112
WC vs MC- BP	0.656	0.110	0.021
Gb vs MC- PJ	0.845	0.328	0.276
Gb vs MC-AP	0.653	0.421	0.319
Gb vs MC-BP	0.453	0.586	0.642
MC-PJ vs MC-AP	0.851	0.311	0.453
MC-PJ vs MC-AP	0.679	0.669	.0.786
MC-AP vs MC-BP	0.858	0.136	0.217

Results are expressed as mean±SD; WC=water control; Glibenclamide; MC-PJ= *M. charantia* pulp juice; MC-AP= *M. charantia* aqueous soluble part; MC-BP= *M. charantia* 1-butanol soluble part; One-way ANOVA with post hoc bonferroni was performed as the test of significance.

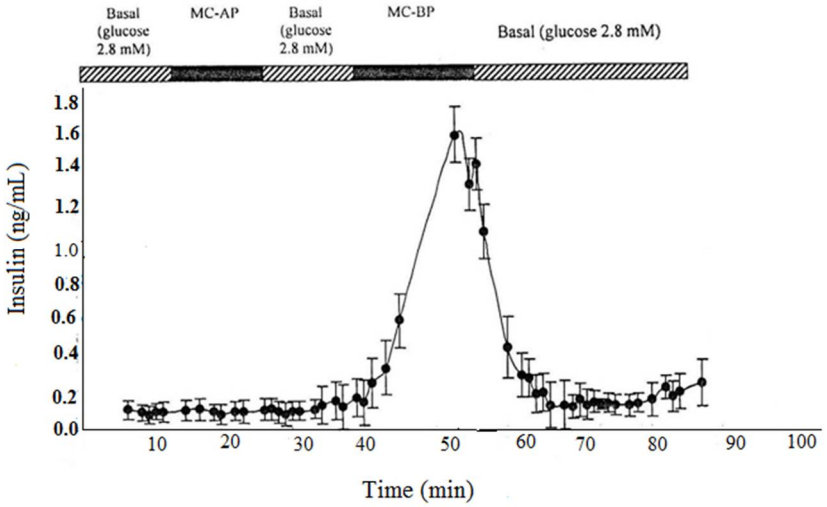

Figure 1. Effects of aqueous part (MC-AP) and 1-butanol soluble part (MC-BP) of *M. charantia* on insulin release from perfused rat pancreas. Each point is the mean±SD of 3 separate experiments. Pancreas was perfused (1 mL/min) with MC-AP and MC-BP at a dose of 1 mg/min, each preceded and followed by basal solution with (2.8 mM glucose).

Table 2. Effect of 1-butanol soluble part of *M. charantia* extract on insulin release from perfused pancreas (peak value) in presence of 11 mM glucose and diazoxide.

Perfusate	Baseline insulin (ng/ml)	Peak insulin (ng/ml)
11 mM glucose	0.308±0.011	2.580±0.173[a]***
11 mM glucose plus MC-BP (n=3)	0.215±0.008	3.595±0.194[b]***
11 mM glucose plus MC-BP plus Diazoxide (n=3)	0.354±0.014	4.686±0.187[b]***

Pancreas was perfused with 11 mM glucose alone; then 11 mM glucose and MC-BP; and then with 11 mM glucose, MC-BP and diazoxide. In each case it was preceded and followed by basal solution (with 2.8 mM glucose). MC-BP= *M. charantia* 1-butanol soluble part. Results are expressed as mean ± SD. [a] vs basal, vs [b]11 mM glucose. ***P<0.001.

Further studies on isolated perfused pancreas were performed to evaluate the possible mechanisms underlying the stimulatory actions of *M. charantia* using non-toxic concentrations of MC-BP. As shown in Table 2, the insulin release induced by 200 µg/mL MC-BP was potentiated by 11 mM glucose (p<0.001) and the effect was not abolished by diazoxide. Rather the effect was potentiated by diazoxide (p< 0.001; Table 2). Furthermore, no obvious inhibitory effect was observed in the presence of verapamil, a Ca^{2+} channel blocker (Figure 2). The enhancement of insulin secretion by MC-BP was further increased by the presence of 11 mM glucose with a phosphodiesterase inhibitor theophylline (p<0.001; Figure 3).

The antidiabetic activity of *M. charantia* has been well reported. However, the mechanism of antihyperglycemic action of this plant has not yet been fully elucidated. This study has examined the insulinotropic effects of two sub-fractions of *M. charantia* using the perfused rat pancreas from type 2 diabetic rats. Prominent insulin-secretory effect of 1-butanol soluble part was noted in the perfused rat pancreas (at basal concentration) whereas the aqueous soluble part of *M. charantia* sub-fraction (MC-AP) showed non-significant increase in insulin secretion. Nontoxic concentration was used to study underlying mechanism of insulin release. MC-BP stimulated basal insulin secretion was enhanced by increasing the glucose concentration over the range 2.8-11 mM.

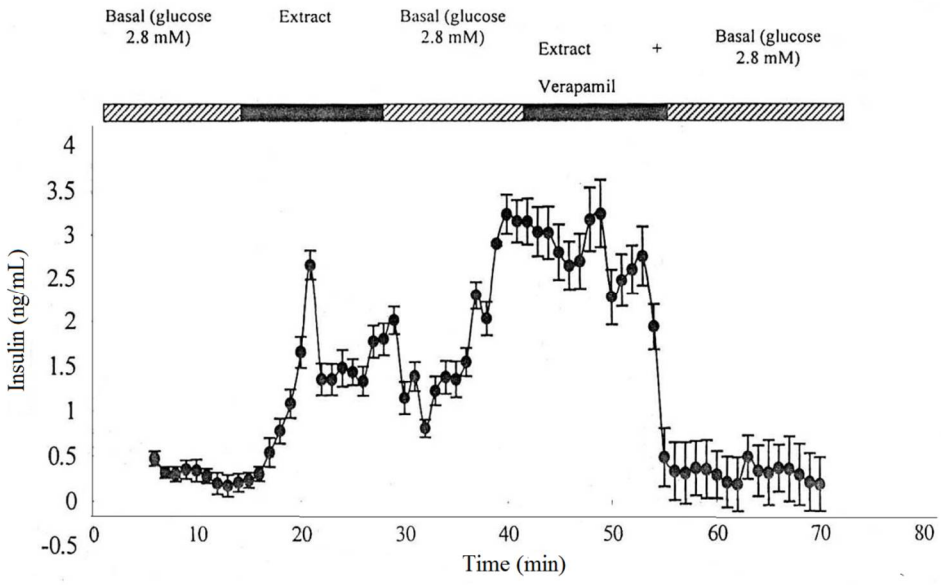

Figure 2. Isolated perfused pancreas study of *M. charantia* extract and change effected by verapamil. Pancreas was perfused with MC-BP; then with MC-BP plus verapamil (50 µM). In each case it was preceded and followed by basal solution (with 2.8 mM glucose).

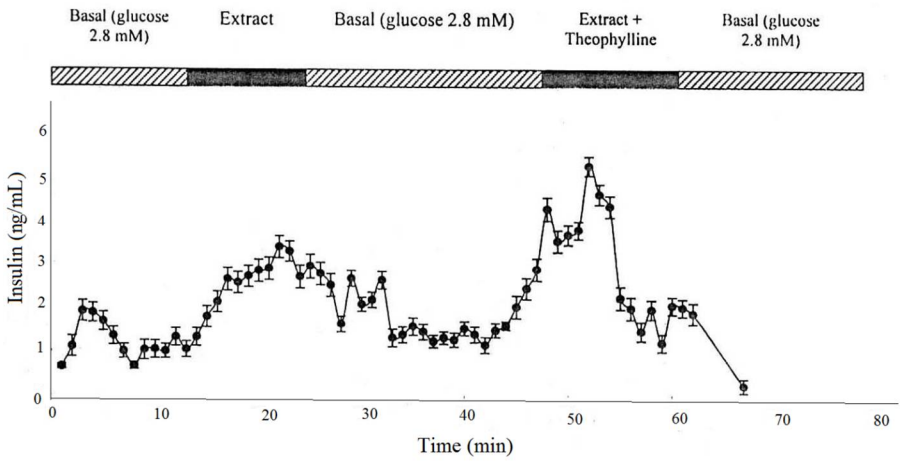

Figure 3. Isolated perfused pancreas study effect of 1-butanol soluble part of *M. charantia* at euglycemic state and change effected by theophylline. Pancreas was perfused with MC-BP; then with MC-BP plus theophylline (10 mM). In each case it was preceded and followed by basal solution (with 2.8 mM glucose).

Additional experiments were performed to assess the effects on insulin secretion in the presence of other modulators that are known to affect secondary-messenger pathways in β-cells. The effect of theophylline (an inhibitor of cyclic AMP phosphodiesterase)[32] was markedly increased by MC-BP in basal solution (with 2.8 mM glucose).

Experiments were also carried out to verify whether ion channels are involved in the stimulatory action of MC-BP. Diazoxide, K^+_{ATP}-channel opener[33], did not inhibit insulin-releasing effect of MC-BP. Similar effects were found with verapamil, a voltage-dependent Ca^{2+} channel blocker[34] that did not abolish the insulin secretory effect of MC-BP.

In conclusion, the obtained results suggest that 1-butanol soluble part of fresh jouice of *M. charantia* fruits has direct insulinotropic action both in basal and glucose stimulated states. Its activity does not seem to be associated with any cellular detrimental effect on the β-cells; rather the secretory activity is mediated through specific cell signaling pathway(s). The data also suggest that the insulinotropic action of the extract is, at least partly, mediated by a cAMP dependent pathway.

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