



## Research Article

### ANTIHYPERGLYCEMIC ACTIVITY OF CHARANTIN ISOLATED FROM FRUITS OF *MOMORDICA CHARANTIA* LINN.

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#### ABSTRACT

In recent years, there has been an increased interest in the development of alternative medicines for maintaining glucose homeostasis in diabetes, specifically by screening plant extracts as well as their isolated compounds for their ability to delay or prevent carbohydrate metabolism and absorption. The objective of present study was to undertake *in-vitro* and *in-vivo* studies to generate a stronger biochemical rationale for the management of post prandial hyperglycaemia (PPHG) with charantin. In this study, charantin ( $\beta$ -sitosteryol glucoside) was isolated from *Momordica charantia* Linn. by normal phase column chromatography and screened for the inhibition of key enzymes related to carbohydrate metabolism. Charantin showed mild  $\alpha$ -amylase ( $IC_{50}$  2.71 $\pm$ 0.21 mg/mL) and strong  $\alpha$ -glucosidase ( $IC_{50}$  1.82 $\pm$ 0.15 mg/mL) inhibitory activity. The positive *in-vitro* enzyme inhibition tests paved way for confirmatory *in-vivo* studies. The *in-vivo* studies demonstrated that charantin (20 mg/kg, b.w.) given orally significantly ( $P<0.01$ ) reduced area under curve (AUC) in mice when challenged with oral administration of starch and sucrose separately. The reduction in AUC by charantin was comparable to that of acarbose (10 mg/kg, b.w., p.o.). These studies indicated the potential of charantin to prevent PPHG by inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes.

**Keywords:** *Momordica charantia*, charantin,  $\alpha$ -amylase,  $\alpha$ -glucosidase, hyperglycemia, PPHG.

#### INTRODUCTION

Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. The number of people in the world with diabetes has increased dramatically over the recent years<sup>1,2,3</sup>. The treatment of type 2 diabetes is complicated by several risk factors inherent to the disease. Elevated postprandial hyperglycemia (PPHG) is one of the risk factors<sup>4</sup>. One important factor to result in PPHG is the fast uptake of glucose in the intestine, in which  $\alpha$ -amylase and  $\alpha$ -glucosidase play important roles due to hydrolysis of polysaccharide and oligosaccharides. Inhibition of these enzymes has beneficial effects on glycemic control in diabetic patients<sup>5</sup>. Glucose homeostasis is also desirable to prevent diabetes related complications like cataract and nephropathy. Therefore, managing PPHG through the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes is an interesting strategy that excludes the involvement of insulin (pancreas). Inhibitors such as acarbose, voglibose and miglitol have been used clinically to control postprandial blood glucose levels in diabetes<sup>6</sup>.

*Momordica charantia* Linn. (Cucurbitaceae) fruits are well known for their beneficial effects in diabetes that are often attributed to its bioactive component charantin<sup>7</sup>. It is commonly known as karela, bitter melon and bitter-gourd. Traditionally, it is considered as useful against diabetes and its related complications<sup>8-10</sup>. The fruits reduce elevated blood glucose level<sup>11</sup>, improve glucose tolerance<sup>12</sup>, decrease insulin resistance<sup>13</sup>, and increase the mass of  $\beta$ -cells in pancreas<sup>14</sup>. Charantin, the main bioactive component, has been found to be more potent than oral hypoglycemic agent tolbutamide in alloxan-induced diabetic

rabbits<sup>15</sup>. Charantin is also reported to have antihyperglycemic and antifertility effects in rabbits<sup>16</sup>. There are no previous studies of evaluation of the role of charantin in glucose homeostasis. Hence, the present study aimed to establish the effectiveness of charantin in achieving glucose homeostasis via inhibition of carbohydrate metabolizing enzymes by *in-vitro* and *in-vivo* studies.

#### MATERIALS AND METHODS

##### Plant Material

Fresh unripe fruits of *M. charantia* were collected from local vegetable market. A voucher specimen (PRL/JH/11/03) was deposited in Phyto-pharmaceuticals Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India.

##### Chemicals

$\alpha$ -Amylase,  $\alpha$ -glucosidase, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) and 3,5-dinitrosalicylic acid (DNS) were purchased from SRL, Bangalore, India. Acarbose was obtained as gift sample from Medley Pharmaceutical Ltd. Jammu, India. All other solvents and chemicals were of analytical grade.

##### Isolation of Charantin

The fruits of bitter gourd were cleaned and sliced into small pieces and dried in an oven at 45 °C. Dried sample was pulverized

to a coarse powder using a grinder. About 1.5 kg of powder was extracted in Soxhlet apparatus for 72 hr with methanol (5 L). The extract was filtered and evaporated under reduced pressure at 50 °C in a rotary evaporator (Buchi, Switzerland). The total methanolic residue (280.37 g, 18.69 % w/w) was suspended in water (1 L) and then sequentially fractionated with hexane and ethyl acetate thrice (1 L, each). The ethyl acetate fraction (32 g; 2.01 % w/w) was subjected to normal phase flash column chromatography using silica gel (60-120 mesh). The column was eluted with chloroform: methanol (50:50 v/v) and 15 fractions of 250 ml each were collected. The fractions were subjected to TLC to check their homogeneity. Chromatographically identical fractions were combined and concentrated. Fractions 1-5 were pooled and yielded crude powder of charantin on keeping. Melting point, mass and other spectroscopic data were recorded for characterization and comparison. m.p.: 136-138°C.; UV  $\lambda_{\text{max}}$  (MeOH): 205 nm; IR  $\nu_{\text{max}}$  (KBr): 3430 (OH), 1620 (unsaturation), 1375 (C-O-C), 1121, 1055, 955  $\text{cm}^{-1}$ ; +ve EIMS  $m/z$ : 576 ( $\text{C}_{35}\text{H}_{60}\text{O}_6$ );  $^1\text{H}$  and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): data comparable.

### In-Vitro Enzyme Inhibition Assays

The  $\alpha$ -amylase inhibitory activity was carried out as per the method given by Ahamad et al.,<sup>1</sup> with suitable modification. Briefly, 40  $\mu\text{L}$  of charantin or acarbose (20 mM sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride) was premixed with 200  $\mu\text{L}$  of  $\alpha$ -amylase solution (1.0 U/mL in phosphate buffer pH 6.9), and incubated at 25 °C for 30 min. After pre-incubation, 400  $\mu\text{L}$  of 0.25 % starch solution in the phosphate buffer (pH 6.9) was added to each tube to start the reaction. The reaction was carried out at 37 °C for 5 min and terminated by addition of 1.0 mL of the DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were then kept over a boiling water bath for 10 min and cooled to room temperature. The reaction mixture was then diluted by making up the volume to 10 ml by distilled water and absorbance (A) was measured at 540 nm. Control incubations representing 100% enzyme activity were conducted in a similar way by replacing test sample with buffer. For blank incubation enzyme and test solutions were replaced by buffer solution and absorbance recorded.

The  $\alpha$ -glucosidase inhibitory activity was assessed by the standard method as described by Ahamad et al.,<sup>1</sup> with slight modifications. Briefly, a volume of 60  $\mu\text{L}$  of sample solutions in DMSO charantin or acarbose in DMSO and 50  $\mu\text{L}$  of 0.1 M phosphate buffer (pH 6.8) containing  $\alpha$ -glucosidase solution (0.2 U/mL) was incubated in 96 well plate at 37 °C for 20 min. After pre-incubation, 50  $\mu\text{L}$  of 5 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 °C for another 20 min. Then the reaction was stopped by adding 160  $\mu\text{L}$  of 0.2 M  $\text{Na}_2\text{CO}_3$  into each well, and absorbance (A) recorded at 405 nm by micro-plate reader and compared to a control which had 60  $\mu\text{L}$  of buffer solution in place of the test sample. For blank incubation enzyme and test solutions were replaced by buffer solution and absorbance recorded.

The percent inhibition of enzymes was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

where  $A_{\text{control}}$ ,  $A_{\text{test}}$ ,  $A_{\text{background}}$  are defined as the absorbance of 100% enzyme activity, test sample with the enzyme and test sample without the enzyme, respectively.

The concentration of inhibitors required for inhibiting 50% of enzyme activity under assay conditions was defined as  $\text{IC}_{50}$  value.

## Confirmatory In-Vivo Studies

### Animals

Wistar albino mice (30-40 g) were obtained from Central Animal Facility, Jamia Hamdard and maintained under controlled conditions of illumination (12h light/12h darkness) and temperature (20-25 °C). They were housed under ideal laboratory conditions, maintained on standard pellet diet (Lipton rat feed Ltd., Pune, India) and water *ad libitum* throughout the experimental period. Animals were acclimatized to the conditions before start of the experiments. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi, India (Approval no. JH/CAHF/173/CPCSEA/2012/926).

### Oral Carbohydrate Challenge Tests

Mice were fasted overnight for 12 h but had free access to water. The animals were randomly divided into seven groups consisting of six mice in each group ( $n = 6$ ). Group I served as normal control which received 1 mL/kg b.w. vehicle (0.5% CMC in distilled water). For the oral starch tolerance test, Group II served as starch challenge control that received starch (3 g/kg, b.w.). Group III received acarbose as a standard drug (10 mg/kg, b.w.) while as group IV was administered charantin (20 mg/kg, b.w.). Treatment groups III and IV were fed starch (3 g/kg, b.w.) after 20 min of treatment. For oral sucrose tolerance test, group V served as sucrose challenged control that received sucrose (4 g/kg, b.w.). Treatment groups VI and VII received acarbose (10 mg/kg, b.w.) and charantin (20 mg/kg, b.w.), respectively followed by sucrose (4 g/kg, b.w.) after 20 min of the treatment. Blood was withdrawn from the tail vein at 0, 30, 60, 90 and 120 min after carbohydrate challenge. Blood glucose level (BGL) was measured using one-touch glucometer (my life Pura, Switzerland). The AUC was calculated using Trapezoidal method<sup>17</sup>.

### Statistical Analysis

Values are expressed as mean  $\pm$  SD. Statistical significance was calculated by using one-way analysis of variance followed by Dunnett's t-test. The values were considered significantly different when  $P < 0.05$ .

## RESULTS

### Isolation of Charantin

Charantin ( $\beta$ - Sitosteryl glucoside) was isolated by eluting the column with chloroform: methanol (50:50 v/v), as colourless crude powder from the fractions 1-5. The powder was recrystallized from methanol that gave colourless amorphous powder of charantin (215 mg, 0.028 % yield) with m.p. 136-138°C. The structure of charantin (Figure 1) with a molecular formula  $\text{C}_{35}\text{H}_{60}\text{O}_6$  and molecular weight 576 amu, was confirmed by comparing with previously reported data<sup>18</sup>.

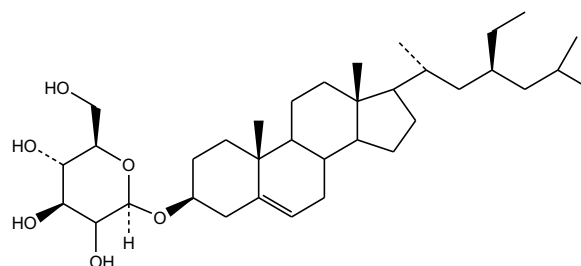
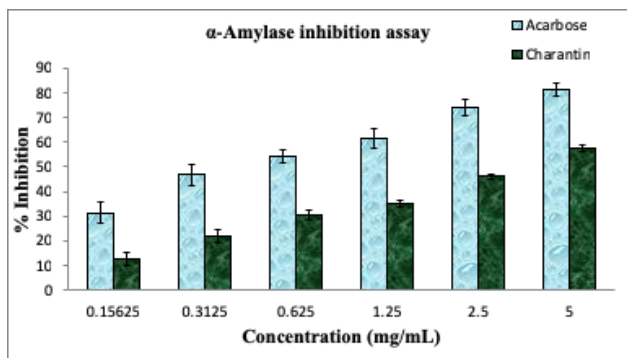


Figure 1: Chemical structure of Charantin ( $\beta$ - Sitosteryl glucoside)

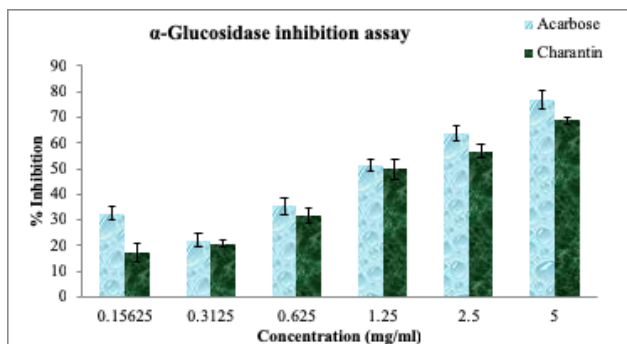
### Enzyme Inhibition by Charantin

The charantin showed concentration dependant  $\alpha$ -amylase inhibition that varied from 56.23 $\pm$ 2.48 to 11.76 $\pm$ 1.48 % for 5 to 0.15 mg/mL, respectively. Acarbose showed a concentration dependant response that varied from 81.33 $\pm$ 2.31 to 31.29 $\pm$ 4.35 % for 5 to 0.15 mg/mL, respectively. Figures 2 showed the percentage inhibition of  $\alpha$ -amylase by charantin and acarbose. The IC<sub>50</sub> values for charantin and acarbose were found as 2.71 $\pm$ 0.21 and 0.42 $\pm$ 0.02 mg/mL, respectively.



**Figure 2: Inhibitory activity of acarbose and charantin against  $\alpha$ -amylase**  
(Data were presented as mean of triplicate determinations  $\pm$  SD)

The results of *in-vitro*  $\alpha$ -glucosidase inhibitory study are depicted in Figure 3. The charantin showed concentration dependant  $\alpha$ -glucosidase inhibition varying from 63.91 $\pm$ 1.45 to 14.16 $\pm$ 4.81 % for 5 to 0.15 mg/mL, respectively. Acarbose showed a concentration dependant response that varied from 76.82 $\pm$ 4.48 to 16.81 $\pm$ 2.68 % for 5 to 0.15 mg/mL, respectively. The IC<sub>50</sub> values for charantin and acarbose were found as 1.82 $\pm$ 0.15 and 1.41 $\pm$ 0.17 mg/mL, respectively.

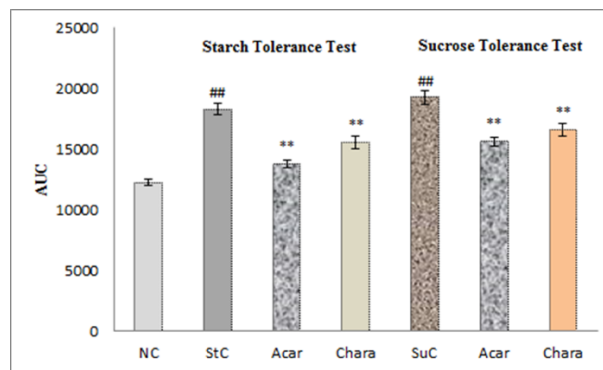


**Figure 3: Inhibitory activity of acarbose and charantin against  $\alpha$ -glucosidase**  
(Data were presented as mean of triplicate determinations  $\pm$  SD)

### Antihyperglycemic Activity

*In-vivo* studies revealed that oral administration of starch (3 g/kg, b.w.) resulted significant (P<0.01) increase in blood glucose level in mice. Pre-treatment with charantin (20 mg/kg, b.w.) decreased BGL significantly (P<0.05) after 30 min of starch challenge to mice. Acarbose (10 mg/kg, b.w.) in mice also produced a significant (P<0.01) blood glucose lowering response after 30 min (Table 1). The treatment with acarbose and charantin showed

a significant (P<0.01) decrease in AUC in comparison to starch control group (Figure 4). Oral administration of sucrose (4 g/kg, b.w.) resulted significant (P<0.01) increase in BGL of mice. Pre-treatment with charantin (20 mg/kg, b.w.) decreased BGL significantly (P<0.05) compared to sucrose challenge to mice. Acarbose (10 mg/kg, b.w.) also produced a significant (P<0.01) blood glucose lowering response after 30 min (Table 1). The treatment with acarbose and charantin showed a significant (P<0.01) decrease in AUC in comparison to sucrose control group (Figure 4).



**Figure 4: AUC of carbohydrate challenged mice after treatment with acarbose and charantin**

Data were expressed as mean $\pm$ SD, n=6, \*\*P<0.01, test groups vs respective carbohydrate controls; ##P<0.01 carbohydrate controls vs normal control (NC). Treatment groups StC: starch control (3 g/kg, b.w.); SuC: sucrose control (4 g/kg, b.w.); Acar: acarbose (10 mg/kg, b.w.) and Chara: charantin (20 mg/kg, b.w.)

### DISCUSSION

*M. charantia* has a long history of human use in traditional medicine throughout the world. Bitter-gourd fruits are well known for their beneficial effects in diabetes that are often attributed to its bioactive component charantin.<sup>9,15</sup> The inhibitors of carbohydrate metabolizing enzymes such as acarbose significantly affect the activities of both these enzymes. The non-specificity of action of these inhibitors results in flatulence that is due to an excessive inhibition of  $\alpha$ -amylase leading to abnormal bacterial fermentation of undigested carbohydrates.<sup>6</sup> Therefore, agents with comparatively more inhibitory activity against  $\alpha$ -glucosidase than against  $\alpha$ -amylase will be helpful to overcome this challenge.<sup>1</sup> Our results indicate that charantin showed stronger inhibition against  $\alpha$ -glucosidase (IC<sub>50</sub> 1.82 $\pm$ 0.15 mg/mL) than  $\alpha$ -amylase (IC<sub>50</sub> 2.71 $\pm$ 0.21 mg/mL). The inhibition for  $\alpha$ -glucosidase by charantin (IC<sub>50</sub> 1.85 $\pm$ 0.18 mg/mL) was less than that of acarbose (IC<sub>50</sub> 1.45 $\pm$ 0.09 mg/mL). In *in-vivo* experiments, pre-treatment with charantin restricted the blood glucose excursions and decreased both peak BGL and AUC in starch and sucrose challenged mice and the effect was comparable to acarbose. Charantin seemed to inhibit the carbohydrate metabolizing enzymes in the brush border of the small intestine. It successfully delayed the carbohydrate absorption. The retardation and delay of carbohydrate metabolism and absorption by charantin offers a prospective therapeutic approach for the management of PPHG for pre-diabetics or who have blood glucose levels only slightly above the level considered serious for management through oral hypoglycemics or insulin.

**Table 1: Effect on blood glucose level of charantin and acarbose in carbohydrate challenged mice**

Group	Blood glucose level (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
<b>Starch tolerance test</b>					
NC	101.4±1.20	100.8±1.07	102±1.14	103±0.45	102±1.09
StC	100.6±1.54	170.4±1.86 <sup>##</sup>	161.8±1.93 <sup>##</sup>	154.6±1.07 <sup>##</sup>	146±1.64 <sup>##</sup>
Acar	98.4±0.24	130.8±2.27 <sup>**</sup>	115.4±1.33 <sup>**</sup>	109.8±0.73 <sup>**</sup>	106.4±0.75 <sup>**</sup>
Chara	96.8±1.65	151.4±1.96 <sup>*</sup>	141±3.36 <sup>**</sup>	134.4±2.6 <sup>**</sup>	122.6±1.4 <sup>**</sup>
<b>Sucrose tolerance test</b>					
SuC	102.8±0.97	180.6±2.25 <sup>##</sup>	175.2±2.08 <sup>##</sup>	160.6±2.76 <sup>##</sup>	148.8±1.07 <sup>##</sup>
Acar	102.2±1.2	143.8±1.66 <sup>**</sup>	139.8±1.16 <sup>**</sup>	128.8±1.56 <sup>**</sup>	115.6±0.68 <sup>**</sup>
Chara	100.6±0.75	172.4±2.98 <sup>ns</sup>	156.8±3.34 <sup>**</sup>	136.2±1.66 <sup>**</sup>	127.2±0.80 <sup>**</sup>

Data were expressed as mean±SD, n=6, \*P<0.05, \*\*P<0.01, test groups vs respective carbohydrate controls; <sup>##</sup>P<0.01 carbohydrate controls vs normal control (NC). Treatment groups StC: starch control (3 g/kg, b.w.); SuC: sucrose control (4 g/kg, b.w.); Acar: acarbose (10 mg/kg, b.w.) and Chara: charantin (20 mg/kg, b.w.)

**CONCLUSION**

The present study demonstrated the role of charantin in inhibiting α-amylase and α-glucosidase enzymes. Charantin was effective in the achieving stricter glycemic control in carbohydrate challenged mice through the inhibition of carbohydrate metabolizing enzymes. The present study proves the traditional use of *M. charantia* in diabetes mellitus.

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