www.sospublication.co.in Journal of Advanced Laboratory Research in Biology

We- together to save yourself society e-ISSN 0976-7614

Volume 3, Issue 3, July 2012 Research Article

Antidiabetic Effects of *Momordica charantia* (Karela) in Male long Evans Rat

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Abstract: The hypoglycemic effect of *Momordica charantia* (Karela) has been reported from many laboratories. To our knowledge, the underlying biochemical mechanism of action of this important clinical effect has not been reported. During the course of investigation of this aspect of the herbal fruit, it was reported from our laboratory that ethanolic extract of *Momordica charantia* suppressed gluconeogenesis in normal and streptozotocin (STZ) induced diabetic rats by depressing the hepatic gluconeogenic enzymes fructose-1,6-bisphosphatase and glucose-6-phosphatase. The herbal extract had also enhanced the activity of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of hexose monophosphate shunt (a pathway for the oxidation of glucose).

Keywords: *Momordica charantia*, Antidiabetic effect, Streptozotocin, Hexose monophosphate shunt.

1. Introduction

According to World Health Organization (WHO), medicinal plants are an accessible, affordable and culturally appropriate source of primary health care for more than 80% of Asia's population [1]. Despite all the progress in synthetic chemistry and biotechnology, plants are still an indispensable source of medicinal preparations, both preventive and curative. Hundreds of species are recognized as having medicinal values and many of those are commonly used to treat and prevent specific ailments and diseases [2]. *Momordica charantia* (called bitter melon or bitter gourd in English or Karela in Bangla) is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa and the Caribbean for its edible fruit, which is among the most bitter of all fruits, is one of the well known medicinal plants.

This herbaceous, tendril-bearing vine grows to 5 meters. It bears simple, alternate leaves 4–12cm across, with 3–7 deeply separated lobes. Each plant bears separate yellow male and female flowers. In the Northern Hemisphere, flowering occurs during June to July and fruiting during September to November. As the fruit ripens, the flesh becomes tougher, bitterer, and too distasteful to eat. On the other hand, the pith becomes sweet and intensely red; it can be eaten uncooked in this state and is a popular ingredient in some Southeast Asian salads. When the fruit is fully ripe it turns orange and mushy and splits into segments which curl back dramatically to expose seeds covered in bright red pulp.

The plant contains several biologically active compounds, chiefly momordicin I and II, and cucurbitacin B [3]. The plant contains also several bio-active glycosides (including momordin, charantin, charantosides, goyaglycosides, momordicosides) and other terpenoid compounds (including momordicin-28, momordicinin, momordicilin, momordenol and momordol) [4-8]. It also contains cytotoxic (ribosome-inactivating) proteins such as momorcharin and momordin [9].

In 1962, Lolitkar and Rao extracted from the plant a substance, which they called charantin, which had a hypoglycemic effect on normal and diabetic rabbits [10]. Another principle, active only on diabetic rabbits,

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was isolated by Visarata and Ungsurungsie in 1981 [11]. Bitter melon has been found to increase insulin sensitivity [12]. In 2007, a study by the Philippine Department of Health determined that a daily dose of 100mg/kg of body weight is compared to 2.5mg/kg of the anti-diabetes drug glibenclamide taken twice per day [13]. Tablets of bitter melon extract are sold in the Philippines as a food supplement under the trade name Charantia and exported to many countries [13].

Bitter melon also contains a lectin that has insulin-like activity due to its non-protein-specific linking together to insulin receptors. This lectin lowers blood glucose concentrations by acting on peripheral tissues and similar to insulin's effects in the brain, suppressing appetite. This lectin is likely a major contributor to the hypoglycemic effect that develops after eating bitter melon [14].

2. Materials and Methods

2.1 Experimental design

40 male Long Evans rats, purchased from Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM) and International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), were used to carry out this investigation. All the rats were divided into eight groups of five rats in each group. Group I was normal (fed), Group II was normal (18 hours fasting), Group III was diabetic (Streptozotocin (STZ) induced) control, Group IV was STZ-induced diabetic rats feed with ethanolic extract of *Momordica charantia* (200mg/kg body weight), Group V was STZ-induced diabetic rats treated with oral hypoglycemic drug (Dicon, 25mg/kg body weight), Group VI was STZ-induced diabetic rats treated with rapid acting insulin (Actrapid, 20 units/kg body weight), Group VII was STZ-induced diabetic rats treated together with extract of *Momordica charantia* and drug and Group VIII was STZ-induced diabetic rats treated together with extract of *Momordica charantia* orally and insulin intravenously.

2.2 Source of *Momordica charantia*

Momordica charantia was purchased from Ananda Bazar near Fazlul Haq, Hall of Dhaka University, Dhaka.

2.3 Preparation of 95% ethanolic extract of *Momordica charantia*

The fresh vegetable (1 kg) was thoroughly washed in tap water, cut and the seeds were removed manually. The seedless vegetable was blended with 3 liters of 95% (v/v) ethanol in a blender and kept at room temperature in a flat bottom flask for 48 hours with occasional shaking. The suspension was then filtered and the residue discarded. The alcoholic portion of the filtrate was evaporated in a rotavapor at 40-45°C under reduced pressure. Water was removed by lyophilizer. A yellowish semisolid material was obtained which weighed about 20 grams.

2.4 Induction of diabetes

Streptozotocin (STZ), an anti-tumor agent obtained from the fungus *Streptomyces achromogenes*, is widely used to produce diabetes in experimental animals. It induces diabetes by selectively destroying the β-cells of the islets of Langerhans of the pancreas which secrete insulin. There are some other diabetogenic agents such as alloxan, which also induces diabetes by damaging insulin-secreting cells. In our experiments, STZ was used to induce diabetes as the mortality rate is lower with this drug. STZ was dissolved in physiological saline previously adjusted to pH 4.3 with 0.05M citric acid. This solution was administered by intravenous injection (through tail vein) to 18 hrs fasted rats at a dose of 60mg/kg body weight.

2.5 Oral administration of *Momordica charantia*

2.0g of semisolid alcohol-soluble material of ethanolic extract of *Momordica charantia* obtained was dissolved in water and this suspension was fed orally to the experimental rats at a dose of 200mg/kg body weight.

2.6 Collection of blood tissue samples

Before and after inducing the rats with STZ to become diabetic, blood was drawn by cutting the end of the tail (by tail blotting) using surgical scissors. After 90 minutes of oral administration of an aqueous suspension of plant extract, blood was collected by sacrificing the rats using a scalpel. Na-heparin was used as an anticoagulant and NaF was used as glycolysis inhibitor. Before cutting the tail end, the tail of each rat was kept in warm water (40°C) for 30 seconds and blood was drawn immediately into a small test tube. Livers were surgically removed immediately after sacrificing the animals and taken into glass beakers containing ice-cold saline.

2.7 Storage of blood sample and liver sample

Some portion of blood was immediately used to estimate glucose levels. Other portions of blood were stored at 4°C until used. Livers were stored at -4°C.

2.8 Estimation of blood glucose

For glucose estimation, a reagent kit, supplied from Randox Laboratories Ltd., was used. Blood glucose was estimated by the glucose oxidase-peroxidase (GOD-POD) methods of Trinder.

2.9 Assay of hepatic glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase activity in hepatocytes was assayed by the method of Lohr and Waller. A slight modification of this procedure was

made where Triethanolamine buffer was replaced by Tris-HCl buffer.

2.10 Assay of hepatic fructose-1,6-bisphosphatase

Fructose-1,6-bisphosphatase activity in hepatocytes was assayed by the method of Pogell and McGilvery in which inorganic phosphate liberated from Fructose-1,6-bisphosphate was determined. In the present work, the procedure is slightly modified.

2.11 Assay of hepatic glucose-6-phosphatase

Hepatic glucose-6-phosphatase was assayed by the method of Baginsky *et al*., which involves the estimation of inorganic phosphate liberated from glucose-6-phosphate by this enzyme. In the present work, the procedure was slightly modified.

3. Results and Discussion

It was shown from our experiments that oral administration of the ethanolic extract of *Momordica charantia* (Karela) to rats lowered blood glucose level by suppressing gluconeogenesis by inhibition of the key hepatic gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-bisphosphate dehydrogenase and stimulating glucose oxidation by the pentose phosphate pathway through activation of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of this pathway. These mechanisms of action of the extract were shown only at the enzymatic levels. But no investigation was carried out on the mechanism of action of the extract at the hormonal levels.

In an effort to shed light on the hypoglycemic effects of the extract, the current investigation was undertaken. For this, both insulin and the well known oral hypoglycemic agent namely glibenclamide (locally known as dicon) were administrated with or without the herbal extract of STZ induced diabetic rats to examine this insulin-like effect of the herbal extract.

It is clear from the results presented in Table 3.1 that the levels of two rates limiting enzymes of the hepatic gluconeogenic pathway, glucose-6-phosphatase and fructose-1,6-bisphosphatase, are increased in STZ induced diabetic condition by 71.29% and 52.58%, respectively. Oral administration of 95% of ethanolic extract decreased significantly the activity of enzymes by 40.54% (Table 3.4) and 40.49% (Table 3.3). Dicon and insulin showed stronger effect the corresponding values in percent were 54.05, 48.0 and for insulin were 59.73, 53.37. The effect of extracts was not significant ($p<0.5$) in dicon and insulin-treated rats. Possible mechanism of inhibition of the enzymes may be explained as follows:

STZ is known to destroy the β-cells of islets of Langerhans of the pancreas. So, participation of insulin could be excluded. The extract might act by decreasing glucagon secretion or secondarily by blocking glucagon receptors in the plasma membrane. But this is not consistent with our finding because dicon (and insulin secretagogue) which was found to show hypoglycemic activity in STZ induced diabetic rats. Likewise/similarly extract would be expected to act in the same manner as dicon i.e. by secreting insulin in STZ induced rats. The extract might cause this inhibition directly by elevating the level of fructose-2,6-bisphosphate, which is a potent allosteric modulator of fructose-1,6-bisphosphatase. Glucose-6-phosphatase is also an irreversible gluconeogenic enzyme but not a regulatory enzyme. The possible mechanism of its decreased activity due to oral administration of the extract is not clear.

Glucagon and insulin control glycolysis and gluconeogenesis by regulating the level of fructose-2,6-bisphosphate at one of the most important key crossroads of glucose metabolism namely conversion of fructose-6-phosphate to fructose-1,6-bisphosphate. Insulin by virtue of its ability to lower cAMP raises fructose-2,6-bisphosphate which inhibits gluconeogenesis. On the contrary, glucagon by elevating the levels of cAMP lowers the level of fructose-2,6-bisphosphate and stimulates gluconeogenesis. Glucagon binds to its receptors in the plasma membrane and active the cAMP cascade which in turn produces active protein kinase that phosphorylates two enzymes phosphofructokinase-II and fructose-2,6-bisphosphatase. This phosphorylation inhibits phosphofructokinase-II and stimulates fructose-2,6-bisphosphatase resulting in reduced levels of fructose-2,6-bisphosphate. Since fructose-2,6-bisphosphate is a potent allosteric positive modulator of phosphofructokinase-II and potent inhibitor of fructose-2,6-bisphosphate. The net effect of lowered levels of fructose-2,6-bisphosphate would be and onset of gluconeogenesis and inhibition of glycolysis.

Table 2.1. Body weight (g) and blood glucose (mmol/l) levels of normal and streptozotocin (STZ) induced diabetic rats.

Parameters assayed			Normal (fed)	Normal (18hrs fasting rats)	Diabetic
Body weight (g)	Before STZ injection	Mean ± S.D.	186.2 ± 12.83	179.7 ± 12.18	-
	After STZ injection	Mean ± S.D.	-	-	157.8 ± 16.98
		Effect (%)	-	-	-11.88
		Sig. (*p*)	-	-	*p*<0.05
Blood glucose level (mmol/l)	Before STZ injection	Mean ± S.D.	6.34 ± 0.49	4.08 ± 0.18	-
	After STZ injection	Mean ± S.D.	-	-	16.20 ± 0.90
		Effect (%)	-	-	+297
		Sig. (*p*)	-	-	

Here, "+" indicates increase, "-" indicates decrease.

Table 3.1. Hepatic glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1,6-bisphosphatase levels of normal and STZ induced rats.

Parameters assayed		Normal (18hrs fasting rats)	Diabetic
Glucose-6-phosphatedehydrogenase level (units/mg of protein)	Mean ± S.D.	355.17 ± 9.97	302.4 ± 10.46
	Effect (%)	-	-14.84
	Sig. (p)	-	p < 0.001
Glucose-6-phosphatase level (Units/mg of protein)	Mean ± S.D.	2.16 ± 0.086	3.70 ± 0.193
	Effect (%)	-	+71.29
	Sig. (p)	-	
Fructose-1,6-bisphosphatase level (Units/mg of protein)	Mean ± S.D.	0.42 ± 0.07	0.65 ± 0.06
	Effect (%)	-	+52.58
	Sig. (p)	-	p < 0.001

Here, "+" indicates increase, "-" indicates decrease.

Table 3.2. Blood glucose levels (mmol/l) of streptozotocin-induced diabetic rats (before/after treatment)

	Treatment	Mean±S.D.	Effect (%)	Sig. (p)	Sig *p* (Dicon vs. Extract + Dicon)	Sig *p* (Insulin vs. Extract + Insulin)
Diabetic (untreated)	STZ injection	16.20 ± 0.90				
Diabetic (treated)	Extract of *Momordica charantia* (200mg/kg body wt.)	12.90 ± 1.15	-20.37	p<0.001		
	Dicon (25mg/kg body wt.)	3.92 ± 0.56	-75.80	-		
	Insulin (20units/kg body wt.)	2.82 ± 0.31	-82.59	-		
	Extract (200mg/kg body wt.) + Dicon (25mg/kg body wt.)	6.42 ± 0.95	-60.37	-	p<0.001	
	Extract (200mg/kg body wt.) + Insulin (20units/kg body wt.)	4.22 ± 0.77	-73.59	-		p<0.01

Here, "+" indicates increase, "-" indicates decrease.

Table 3.3. Hepatic fructose-1,6-bisphosphatase (units/mg of protein) levels of streptozotocin induced diabetic rats (before/after treatment).

	Treatment	Mean±S.D.	Effect (%)	Sig. (*p*)	Sig *p* (Dicon vs. Extract + Dicon)	Sig *p* (Insulin vs. Extract + Insulin)
Diabetic (untreated)	STZ injection	0.65 ± 0.061	-	-		
Diabetic (treated)	Extract of *Momordica charantia* (200mg/kg body wt.)	0.388 ± 0.046	-40.49	p<0.001		
	Dicon (25mg/kg body wt.)	0.338 ± 0.037	-48.00	-		
	Insulin (20units/kg body wt.)	0.304 ± 0.043	-53.37	-		
	Extract (200mg/kg body wt.) + Dicon (25mg/kg body wt.)	0.360 ± 0.047	-44.48	-	p<0.50	
	Extract (200mg/kg body wt.) + Insulin (20units/kg body wt.)	0.320 ± 0.022	-50.92	-		p<0.50

Here, "+" indicates increase, "-" indicates decrease

Table 3.4. Hepatic glucose-6-phosphatase (units/mg of protein) levels of streptozotocin-induced diabetic rats (before/after treatment).

	Treatment	Mean ± S.D.	Effect(%)	Sig. (*p*)	Sig *p* (Dicon vs. Extract +Dicon)	Sig *p* (Insulin vs. Extract + Insulin)
Diabetic (untreated)	STZ injection	3.70 ± 0.193	-	-		
Diabetic (treated)	Extract of *Momordica charantia* (200mg/kg body wt.)	2.20 ± 0.035	-40.54	p<0.001		
	Dicon (25mg/kg body wt.)	1.70 ± 0.108	-54.05			
	Insulin (20units/kg body wt.)	1.49 ± 0.019	-59.73			
	Extract (200mg/kg body wt.) + Dicon (25mg/kg body wt.)	1.84 ± 0.143	-50.27		p<0.5	
	Extract (200mg/kg body wt.) + Insulin (20units/kg body wt.)	1.56 ± 0.08	-57.83			p<0.1

Here, "+" indicates increase, "-" indicates decrease

Table 3.5. Hepatic glucose-6-phosphate dehydrogenase (units/mg of protein) levels of streptozotocin-induced diabetic rats (before/after treatment).

	Treatment	Mean ± S.D.	Effect (%)	Sig. (p)	Sig *p* (Dicon vs. Extract + Dicon)	Sig *p* (Insulin vs. Extract + Insulin)
Diabetic (untreated)	STZ injection	302.45 ±10.46	-	-		
Diabetic (treated)	Extract of *Momordica charantia* (200mg/kg body wt.)	388.21 ± 21.76	+28.45	p<0.001		
	Dicon (25mg/kg body wt.)	478.32 ± 21.65	+58.15			
	Insulin (20units/kg body wt.)	499.28 ±26.64	+65.08			
	Extract (200mg/kg body wt.) + Dicon (25mg/kg body wt.)	455.08 ± 22.26	+50.46		p<0.5	
	Extract (200mg/kg body wt.) + Insulin (20units/kg body wt.)	489.49 ± 18.66	+61.84			p<0.5

Here, "+" indicates increase, "-" indicates decrease

Form the above discussion it appears that the hypoglycemic effect caused by *Momordica charantia* is partially mediated through the decrease production of glucose by the process of gluconeogenesis in the liver.

Furthermore, it is evident from the results represented in Table 2.1, 3.1, 3.2 and 3.5 STZ diabetes elevated the blood glucose level by 297% and depressed the activity of liver glucose-6-phosphate dehydrogenase by 14.84% ($p<0.001$). The ethanolic extract of *Momordica charantia* depressed the level of glucose by 20.37% ($p<0.001$) and elevated the activity of hepatic glucose-6-phosphate dehydrogenase by 28.45% ($p<0.001$). Dicon and insulin showed the similar effects qualitatively but much larger quantitatively.

The possible mechanisms of this hypoglycemic effect of extract in rats may be explained as follows:

a) Suppression of the key hepatic gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-bisphosphatase. Due to the depressed activity of these enzymes, the glucose synthesis would decrease and as a result, blood glucose level would be reduced.
b) Elevation in the activity of glucose-6-phosphate dehydrogenase, the hexose monophosphate (HMP) shunt enzyme. The increased activity of this regulatory enzyme of the HMP pathway reflects an increased glucose oxidation through this pathway and thus contributes partially to the overall blood glucose decreasing effect of the extracts in diabetic rats.

4. Conclusion

From the results described above, it is clear that the effect of the extract on the parameters assayed is significant in the absence of dicon or insulin while the effect is insignificant in the presence of them (dicon and insulin). That is, the effect of extract, dicon or insulin-treated animals are non-additive. The results that are described and discussed above suggest that the active hypoglycemic principle(s) in the herbal extract is/are insulin-like, though less potent, in its anti-diabetic properties.

Acknowledgment

We are extremely thankful to the Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh, for providing us laboratory facilities.

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