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Antidiabetic activity of *Momordica charantia* seeds on streptozotocin induced diabetic rats

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Received March 22, 2004, accepted July 8, 2004

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Pharmazie 60: 383–387 (2005)

The present study was aimed to evaluate the hypoglycemic efficacy in an aqueous extract of seeds of two varieties, namely a country and a hybrid variety of *Momordica charantia* (MCSEt1 and MCSEt2) respectively in streptozotocin (STZ) induced diabetic rats. STZ-induced diabetic rats were treated with aqueous extracts of MCSEt1 and t2 for a period of 30 days. MCSEt1 and t2 extract treatment to diabetic rats resulted in a significant reduction in blood glucose, glycosylated hemoglobin, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase and glycogen phosphorylase, and a concomitant increase in the levels of hemoglobin, glycogen and activities of hexokinase and glycogen synthase. These results clearly show the antidiabetic properties of *Momordica charantia*. Both the varieties showed safe and significant hypoglycemic effects which were more pronounced in MCSEt1 compared to MCSEt2 and glibenclamide.

1. Introduction

Momordica charantia (MC), commonly referred to as bitter gourd or karela, is a climber, belongs to the Cucurbitaceae family and is commonly consumed as a vegetable in India as it is very cheap and available throughout the year. Different parts of this plant have been used in the Indian system of medicine for certain disorders, such as diabetes, atherosclerosis and other complications. (Ganguly et al. 2000; Jayasooriya et al. 2000; Ahmed et al. 2001; Toshihiro Miura et al. 2004; Virdi et al. 2003; Ali et al. 1993). The clinical use of the plant is supported mostly by anecdotal reports and its claimed medicinal activities have not yet been investigated in controlled experiments. The purpose of this study was to clarify, whether the seed varieties possess any anti-diabetic properties in experimental models and to rationalize their therapeutic use.

2. Investigations and results

2.1. Acute toxicity

The acute toxicity testing appeared normal and did not show any visible signs of toxicity like weight loss, excitement, restlessness, respiratory distress, convulsion and coma. No mortality was observed during the test period.

2.2. Change in body weight of control and experimental groups

The Fig. shows the body weight of control and experimental groups of rats. A significant decrease in body weight was observed in streptozotocin (STZ) induced diabetic rats (Group II) when compared to the control rats (Group I). *Momordica charantia* and glibenclamide treated rats (Group III, IV and V) showed a progressive increase in body weight which was near normal at the end of experimental period.

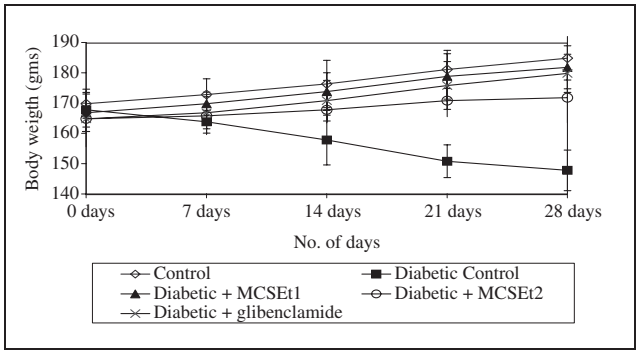

Fig.: Changes in body weight of control and experimental groups of rats

2.3. Oral glucose tolerance test (OGTT) in control and experimental groups

Table 1 shows the blood glucose levels of control and experimental groups of rats after oral administration of glucose. The blood glucose level in the control rats rose to a peak value 60 min after glucose load and decreased to near normal levels after 120 min. In diabetic control rats, the increase in blood glucose was observed after 60 min and remained high over the next 60 min. MC seeds and glibenclamide treated diabetic rats showed a significant decrease in blood glucose concentrations at 60 and 120 min compared with diabetic rats.

Table 1: Oral glucose tolerance test (OGTT) in control and experimental groups

Groups	Fasting	Blood sugar level (mg/dl)			
		30 min	60 min	90 min	120 min
Normal control	79.0 ± 4.5	148.2 ± 1.6	181.2 ± 4.5	132.3 ± 12.8	87.5 ± 4.2
Diabetic control	250.5 ± 2.3*	313.1 ± 1.1*	385.0 ± 4.2*	306.7 ± 3.9*	306.7 ± 3.9*
Diabetic + MCSEt1	88.0 ± 3.2*	167.0 ± 4.2*	145.4 ± 3.9*	118.5 ± 2.5*	93.5 ± 3.8*
Diabetic + MCSEt2	79.1 ± 1.5*	140.7 ± 1.1*	178.5 ± 4.8*	123.7 ± 15.0*	90.3 ± 3.5*
Diabetic + glibenclamide	82.2 ± 1.8*	145.7 ± 5.2*	181.1 ± 3.7*	130.0 ± 12.6*	95.2 ± 4.2*

Values are given as mean ± SD for groups of six animals each
Diabetic control (Group II) was compared with control (Group I)
Experimental groups (Group III, IV & V) were compared with diabetic control (Group II)
Values are statistically significant at *$p < 0.05$

Table 2: Changes in the level of blood glucose, total hemoglobin and glycosylated hemoglobin in control and experimental groups

Groups	Fasting blood glucose (mg/100 ml)	Hemoglobin (g/100 ml)	Glycosylated hemoglobin (mg/gHb)
Normal control	76.2 ± 4.5	12.12 ± 0.60	0.25 ± 0.02
Diabetic control	250.5 ± 5.5*	5.60 ± 0.55*	0.90 ± 0.03*
Diabetic + MCSEt1	85.4 ± 7.1*	10.20 ± 0.73*	0.30 ± 0.04*
Diabetic + MCSEt2	90.5 ± 8.0*	9.90 ± 0.54*	0.48 ± 0.05*
Diabetic + glibenclamide	96.2 ± 6.8*	9.32 ± 0.75*	0.45 ± 0.05*

Values are given as mean ± SD for groups of six animals each
Diabetic control (Group II) was compared with control (Group I)
Experimental groups (Group III, IV & V) were compared with diabetic control (Group II)
Values are statistically significant at *$p < 0.05$

Table 3: Changes in the activities of hepatic hexokinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase of control and experimental groups of rats

Groups	Hexokinase (μmol Glucose-6-phosphate formed/h/mg protein)	Lactate dehydrogenase (μmol pyruvate formed/h/mg protein)	Glucose-6-phosphatase (μmol phosphate liberated/h/mg protein)	Fructose-1, 6-bisphosphatase (μmol phosphate liberated/h/mg Protein)
Normal control	261.7 ± 23.0	248.0 ± 23	1018.3 ± 54	483.1 ± 25
Diabetic control	131.2 ± 12.5*	455.2 ± 4.8*	1824.0 ± 92*	786.2 ± 48*
Diabetic + MCSEt1	260.2 ± 21.7*	250.0 ± 20.0*	1023.5 ± 80*	527.5 ± 31*
Diabetic + MCSEt2	257.0 ± 20.3*	261.2 ± 19.0*	1198.5 ± 75*	548.0 ± 40*
Diabetic + glibenclamide	255.0 ± 18.0*	272.3 ± 19.5*	1409.5 ± 90*	575.0 ± 48*

Values are given as mean ± SD for groups of six animals each
Diabetic control (Group II) was compared with control (Group I)
Experimental groups (Group III, IV & V) were compared with diabetic control (Group II)
Values are statistically significant at *$p < 0.05$

Table 4: Changes in the activities of glycogen, glycogen synthase and glycogen phosphorylase in control and experimental rats

Groups	Glycogen (mg of glucose/g of wet tissue)	Glycogen Synthase (μmol of UDP formed/h/mg Protein)	Glycogen phosphorylase (μmol of phosphate liberated/h/mg protein)
Normal control	55.2 ± 4.5	827.3 ± 50.5	644.7 ± 40.0
Diabetic control	23.1 ± 4.0*	540.1 ± 20.5*	900.2 ± 48.0*
Diabetic + MCSEt1	55.6 ± 2.0*	812.5 ± 45.2*	650.1 ± 32.0*
Diabetic + MCSEt2	52.4 ± 2.7*	801.5 ± 37.2*	640.0 ± 29.6*
Diabetic + glibenclamide	48.2 ± 3.7*	748.5 ± 45.0*	735.6 ± 52.5*

Values are given as mean ± SD for groups of six animals each
Diabetic control (Group II) was compared with control (Group I)
Experimental groups (Group III, IV & V) were compared with diabetic control (Group II)
Values are statistically significant at *$p < 0.05$

2.4. The levels of blood glucose, total hemoglobin, glycosylated hemoglobin of control and experimental groups

Table 2 illustrates the levels of blood glucose, total hemoglobin, glycosylated hemoglobin of control and experimental animals. There was a significant elevation in blood glucose and glycosylated hemoglobin, while the level of total hemoglobin significantly decreased in the diabetic animals. Administration of *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide tended to decrease the levels towards normal values.

2.5. Carbohydrate metabolism

Table 3 shows the changes in the activities of hepatic hexokinase, lactate dehydrogenase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase in control and experimental

rats. The activity of hexokinase decreased while the activity of glucose-6-phosphatase, fructose-1, 6-bisphosphatase and lactate dehydrogenase were increased in STZ-treated diabetic rats as compared to control rats. Administration of *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide increased the activity of hexokinase and decreased the activities of glucose-6-phosphatase, lactate dehydrogenase and fructose-1, 6-bisphosphatase as compared to diabetic rats and the effect was more pronounced in the group of rats administered with *Momordica charantia* extract1 (MCSEt1).

Table 4 shows the changes in hepatic glycogen content and the activities of glycogen synthase and glycogen phosphorylase in control and experimental rats. *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide treated groups restored the enzyme activities and glycogen levels to near normal values and the effect was more pronounced in the group of rats administered with *Momordica charantia* extract 1 (MCSEt1).

3. Discussion

The present study was conducted to evaluate the beneficial effects of two varieties of bitter gourd (*M. charantia*) seed extracts, on STZ-induced diabetic rats.

Preliminary studies revealed the non-toxic nature of MC seeds on normal rats. STZ-induced diabetes is characterized by a severe loss of body weight (Chen and Ianuzzo 1982; Al-Shamaony et al. 1994) and this was also seen in the present study. *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide administration controlled this loss in body weight. Glibenclamide is often used as a standard antidiabetic drug in STZ-induced moderate diabetes to be compared with a variety of hypoglycemic compounds and its effectiveness is known (Andrade-Cetto and Wiedenfeld 2001; Paredes et al. 2001). However, the above drugs did not normalize the body weight completely as it remained less than that of control rats. It is important to note that there were no significant differences in food intake between control and experimental groups of rats. This observation and the decrease in body weight observed in uncontrolled diabetes might be the result of protein wasting due to an unavailability of carbohydrates for utilization as an energy source (Virdi et al. 2003). The treated groups showed increase glucose metabolism and thus enhances body weight in STZ-induced diabetic rats.

Table 1 clearly indicates that the MC seeds led to definitively lower peak blood glucose values 60 min after glucose load and after 120 min. MC seeds might enhance glucose utilization because they significantly decreased the blood glucose level in glucose loaded rats. It is very important to note that seed extracts showed a more pronounced action in the glucose tolerance test.

The possible mechanism by which MC seeds lead to a decrease in blood glucose may be by a potentiation of the insulin effect by increasing either the pancreatic secretion of insulin or its responsiveness. A number of other plants have been reported to exert hypoglycemic activity through insulin-release stimulatory effect (Pari and Umamaheswari 2000; Peungvicha et al. 1998).

The capacity of *Momordica charantia* extracts (MCSEt1 and MCSEt2) in decreasing the elevated blood sugar level to normal glycemic level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. A significant decrease in the level of blood glucose clearly shows that the bitter gourd seed at a dose of 150 mg/kg b.w. proved to be hypoglycemic and par with that of glibenclamide. It may be due to restoration of delayed insulin response or due to an inhibition of intestinal absorption of glucose. Some authors have claimed to find an insulin-like compound from fruits of the plant, which is effective on both subcutaneous and oral administration (Khanna et al. 1981; Kedar and Chakrabarti 1982).

During diabetes the excess of glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin. So the hemoglobin level is decreased in diabetic rats (Alyassin and Ibrahim 1981; Sheela and Augusti 1992). The rate of glycosylation is proportional to the concentration of blood glucose (Monnier and Cerami 1982). The blood sugar at the peak of the glucose tolerance curve correlates with glycosylation (Koeing et al. 1976) and with the improvement of glyceamic control, glycosylated hemoglobin also decreases. Hence estimation of glycosylation of hemoglobin is a well-accepted parameter useful in the management and prognosis of the disease (Chang and Noble 1979). The effect of administration of MC seed extracts and glibenclamide tended to bring the parameters significantly towards normal values.

Diabetes mellitus causes disturbances in the uptake of glucose as well as in glucose metabolism. One of the key enzymes in the catabolism of glucose is hexokinase, which phosphorylates glucose and converts it into glucose-6-phosphate; Phosphorylation of glucose is the first step in glycolysis and severely impaired during diabetes (Vestergaard 1999). The activity of hexokinase decreased significantly in the liver of diabetic rats. Administration of *Momordica charantia* to diabetic rats resulted in a significant reversal in the activity of hepatic hexokinase. The increased activity of hexokinase causes the increase in glycolysis and utilization of glucose for energy production. The decrease in the concentration of blood glucose in diabetic rats treated with *M. charantia* extract may also be as a result of increased liver hexokinase activity thereby increasing glycolysis and increasing utilization of glucose for energy.

Lactate dehydrogenase is the enzyme involved in the final step of anaerobic glycolysis. Increased activity of lactate dehydrogenase in diabetes mellitus was reported (Pozzilli et al. 1997). The lactate dehydrogenase system reflects the NAD^+/NADH ratio, indicated by the lactate/pyruvate ratio of hepatocyte cytosol (Williamson et al. 1967). *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide treated diabetic rats presented significantly restored lactate dehydrogenese activity probably by regulating the NAD^+/NADH ratio by stimulating the oxidation of NADH. Normal lactate dehydrogenase activity is indicative of improved channeling of (pyruvate) glucose for mitochondrial oxidation.

Glucose-6-phosphatase catalyses the final step of glucose production in liver and kidney. Glucose-6-phosphatase and fructose-1,6,bisphosphatase are regulatory enzymes of gluconeogenic pathway. Increased activities of the gluconeogenic enzymes have been observed in the diabetic state. Activation of gluconeogenic enzymes is due to the state of insulin deficiency since under normal conditions insulin functions as a suppressor of gluconeogenic enzyme. Administration of *Momordica charantia* extract and glibenclamide significantly depressed the activities of gluconeogenic enzymes in diabetic rats.

Hepatic glycogen metabolism is one of the processes that play an important role in the maintenance of glucose homeostasis (Hers 1976). The level of liver glycogen is decreased in diabetic conditions (Pugazhenthi et al. 1991).

Glycogen synthase and glycogen phosphorylase are the two key regulator enzymes that catalyze glycogen synthesis (glycogenesis) and degradation (glycogenolysis) respectively. Due to the increased glycogen phosphorylase and decreased glycogen synthase activity the glycogen content was decreased (Roesler and Khandelwal 1986) in diabetic conditions, whereas the *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide treated groups restored the level of hepatic glycogen by means of decreasing the activity of glycogen phosphorylase and increasing the activity of glycogen synthase.

From the above observations it is evident that the *Momordica charantia* seed maintains the glucose homeostasis in STZ-induced diabetes on experimental rats by altering the activities of carbohydrate metabolizing enzymes. *Momordica charantia* extracts (MCSEt1 and MCSEt2) were found to be more effective than glibenclamide. The effect was more pronounced in MCSEt1 compared to MCSEt2. Based on the results of this study, we conclude that the seeds of MC have potential anti-diabetogenic properties and this provides a scientific basis for the utilization of this fruit in folk medicine for the treatment of diabetes. Studies are in progress to elucidate the exact mechanism(s) and components responsible for the medicinal properties.

4. Experimental

4.1. Plant material

Fresh fruits of *Momordica charantia* were procured from a vegetative farm of Chengalpattu, India. Authentication of the plant was carried out by Prof. V. Kaviyarasan, Centre for Advanced Studies in Botany, University of Madras and the voucher specimens of the plants have been retained in the department herbarium (No.1293 & 1294).

4.2. Preparation of seed extracts

The fruits were sliced into two halves and the seeds were selectively collected manually, washed with fresh water and dried in the shade at room temperature. The dried seeds were grounded into fine powder by an electrical mill and mesh (mesh number 50). The powdered seeds were kept in airtight containers at 4 °C until further use. The seed extract was prepared by dissolving a known amount of seed powder in distilled water using a magnetic stirrer. It was then filtered and evaporated to dryness under reduced pressure; the obtained semi solid material was lyophilized and stored in a refrigerator until further use. An aqueous extract, which is the form customarily employed in folk medicine was prepared freshly each time and administered orally. The dosage schedule for the drug was once a day.

4.3. Animals

Male albino rats of Wistar strain weighing around 160–180 g were purchased from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai for the present study. They were acclimatized to animal house conditions, fed with a commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore) and had free access to water. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines.

4.4. Toxicity studies

The effect of oral administration of seed extracts (MCSEt1 & MCSEt2) at a concentration of 150 mg/kg body weight/rat/day for a period of thirty days was carried out to assess the toxic effects of MC seeds. The rats were continuously observed for 2 h after the administration of each dose, to detect changes in various behavioral responses. Any mortality during the experiment and the following seven days was also recorded. The body weight changes were carried out periodically.

4.5. Induction of diabetes

STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents (Junod et al. 1969; Ledoux et al. 1986). Animals after fasting (deprived of food for 16 h had been allowed free access to water), diabetes was induced by intraperitoneal injection of STZ (Sigma, St. Louis, Mo) dissolved in 0.1 M citrate buffer pH 4.5 at a dose of 55 mg/kg body weight (Sekar et al. 1990). The control rats received sodium citrate buffer alone. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 48 h of streptozotocin injection, the fasting blood glucose levels were estimated. STZ treated animals were considered as diabetic when the fasting blood glucose levels observed were above 250 mg/dl. The blood glucose levels and body weight gain were checked periodically.

4.6. Experimental setup

The animals were divided into two sets, one for the evaluation of the glucose tolerance test and a second one for the analysis of biochemical parameters. Each set has five groups with six animals in each group.

Group I: Normal control
Group II: Diabetic control
Group III: Diabetic rats treated with MCSEt1 (150 mg/kg b.w./day) in aqueous solution orally for 30 days.
Group IV: Diabetic rats treated with MCSEt2 (150 mg/kg b.w./day) in aqueous solution orally for 30 days.
Group V: Diabetic rats administered with glibenclamide (600 μg/kg b.w./rat/day) in aqueous medium orally for 30 days (Pari and Umamaheswari 2000)

4.7. Oral glucose tolerance test (OGTT)

After 30 days of treatment, fasting blood samples were taken from all the groups of rats. Four more blood samples were collected at 30, 60, 90, 120 min intervals after administration of glucose at a concentration of 2g/kg body weight, (Joy and Kuttan 1999). All blood samples were collected with potassium oxalate and sodium fluoride solution for the estimation of glucose.

4.8. Biochemical assays

After 30 days of treatment the rats were fasted overnight, and sacrificed by cervical decapitation and the blood was collected using EDTA as anticoagulant. The whole blood was used for the estimation of glucose (Sasaki et al. 1972), hemoglobin (Drabkin and Austin 1932) and glycosylated hemoglobin (Sudhakar Nayak and Pattabiraman 1981; Bannon 1982). For enzymes and glycogen assay, a portion of the liver tissue was dissected out, immediately washed with ice-cold saline and kept at 4 °C. The liver tissue was homogenized in 0.1 M Tris–HCl buffer, pH 7.4 and the supernatant was used for the assay of hexokinase (Brandstrup et al. 1957), glucose-6-phosphatase (Koide and Oda 1959), fructose-1,6-bisphosphatase (Gancedo and Gancedo 1971), lactate dehydrogenase (King 1959), glycogen synthase (Leloir and Goldenberg 1979) and glycogen phosphorylase (Cornblath et al. 1963). Another portion of wet liver was used for the estimation of glycogen content (Morales et al. 1973).

4.9. Statistical analysis

All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± S.D. for six animals in each group.

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