Beneficial Effects of *Momordica charantia* Seeds in the Treatment of STZ-Induced Diabetes in Experimental Rats

Dhanasekar SATISHSEKAR and Sorimuthu SUBRAMANIAN*

Department of Biochemistry and Molecular Biology, University of Madras; Chennai-25, India.

Received December 27, 2004; accepted February 28, 2005

The aim of the present study was to evaluate the effect of the aqueous extract of seeds of two varieties, namely a country and hybrid variety of *Momordica charantia* (MCSEt1 and MCSEt2) on oxidative stress in plasma and pancreas of streptozotocin (STZ) induced diabetic rats. Oral administration of each of the seed extracts at a dosage of 150 mg/kg body weight for 30 d resulted in a significant reduction in plasma glucose, thiobarbituric acid-reactive substances, lipid-hydroperoxides, superoxide dismutase, catalase, glutathione peroxidase and significant improvement in ascorbic acid, reduced glutathione and insulin. The treatment also resulted in a significant reduction in thiobarbituric acid reactive substances, lipid-hydroperoxides, superoxide dismutase, catalase, glutathione peroxidase and significant improvement in reduced glutathione in pancreas of drug treated diabetic rats when compared to the untreated diabetic rats. On the basis of results obtained, it may be concluded that the treatment of *Momordica charantia* seed varieties may effectively normalize the impaired oxidative stress in streptozotocin induced-diabetes than the glibenclamide treated groups.

**Key words** *Momordica charantia*; oxidative stress; streptozotocin; diabetes

Diabetes mellitus is a complex syndrome involving severe insulin dysfunction in conjugation with gross abnormalities in glucose homeostasis and lipid metabolism, which has affecting several millions of population all over the world. The individual with diabetes has a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene and a 2 to 6 fold increased risk of coronary heart disease and ischemic brain damage.

There is considerable evidence on the role of free radicals in the etiology of diabetes and altered antioxidant defences in diabetes. Oxidative stress has been reported to play an important role in diabetes mellitus right from its genesis to the development of microvascular complications. Generation of free radicals by hyperglycemia is related to glucose auto-oxidation. Glucose auto-oxidation has been linked to non-enzymatic glycosylation and glycosylated proteins have been shown to be a source of free radicals.

Prior to 1950's control of diabetes was based entirely on insulin therapy. Unfortunately, some patients developed complications and thus need for some other therapy was realized. Presently control of diabetes mellitus relies on many chemicals and plant extracts. More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only a small number of these have received scientific and medical evaluation to assess their efficacy. There was a gap in proper understanding of medicinal plants for mankind in past because traditional medicines generally lacked scientific explanations.

Many plant extracts and plant products have been shown to reduce oxidative stress significantly, which may be an important property of plant medicines associated with the treatment of several ill fated diseases including diabetes. It has been found that compounds in their natural formulations are more active than their isolated form. Among these *Momordica charantia* is a good example of ignorant folk medicine out stripping scientific understandings in the therapeutic applications is selected for this study. Consumption of bitter gourd (*Momordica charantia*) by diabetic patients is a common practice in India, with the belief that it has a useful hypoglycemic potential.

*Momordica charantia* (MC) LINN, commonly referred to as bittergourd or karela, belongs to the Cucurbitaceae family. It is a climbing plant, cultivated throughout Southern Asia. Its fruits are very cheap and available throughout the year. Immature fruits are used to prepare different dishes for human consumption, while highly matured fruits are considered as not worthy for consumption. There are two varieties of this vegetable based on size and shape. The large variety is long, oblong and pale green in color. The other one is small, little oval and dark green in color. The yield of small variety per plant is much less when compared to large ones; as a result the cost of the small variety is almost thrice when compared to the larger ones. Both the types are bitter in taste. The pulp is blood red or scarlet after dehiscence. The seeds are dappled, flat, thick notched margin, red aril in morphology and it is white color in raw fruits and become red when they are ripe. Different parts of these plants have been used in the Indian system of medicine for a number of ailments besides diabetes. Our previous experimental results were highly encouraging as they revealed that blood glucose level was significantly lowered after oral administration of aqueous extract of *Momordica charantia* seeds in glucose load condition and in streptozotocin induced diabetes. In view of the above considerations, the present study was designed to examine the effect of an aqueous extract from the seeds of *Momordica charantia* on oxidative stress in plasma and pancreas of diabetic rats induced by streptozotocin, and their efficacy was compared with glibenclamide, a standard hypoglycemic drug.

**MATERIALS AND METHODS**

**Chemicals** Streptozotocin was procured from Sigma chemical Co., St. Louis Mo, U.S.A. RIA kit for plasma insulin assay was purchased from Linco research Inc., U.S.A. All other chemicals were of analytical grade.

**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. Kanniyarasam, Centre for Advanced Studies in Botany, University of Madras and the voucher specimens of the plants have been retained in the department herbarium.

**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 shade at room temperature. The dried seeds were ground into fine powder by an electrical mill and mesh (mesh number 50). The powdered seeds were kept in airtight containers in a deep freeze maintained at 4 °C until the time of further use. The seed extract was prepared by dissolving the seed powder in distilled water using a magnetic stirrer. It was then filtered and evaporated to dryness under reduced pressure. An aqueous suspension, which is the form customarily used in folk medicine, was prepared to facilitate easy handling. The drug solutions were prepared freshly each time and administered intragastrically. The dosage schedule for the drug was once a day.

**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 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 Justice and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines (Approval number: 360/01/A/CBCSEA).

**Induction of Diabetes** STZ-induced hyperglycemia has been described as a useful experimental model to evaluate the activity of hypoglycemic agents. After overnight fasting (deprived of food for 16 h but had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) at a dose of 55 mg/kg body weight. The animals were allowed free access to water. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines (Approval number: 360/01/A/CBCSEA).

**Experimental Set Up** The animals were divided into five groups with six animals in each group as follows.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control rats</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control rats</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic rats treated with MCSEt1 (150 mg/kg b.w/d) in aqueous solution orally for 30 d.</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic rats treated with MCSEt2 (150 mg/kg b.w/d) in aqueous solution orally for 30 d.</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic rats administered with glibenclamide (600 μg/kg b.w/rat/d) in aqueous solution orally for 30 d.</td>
</tr>
</tbody>
</table>

After 30 d of treatment, the fasted rats were sacrificed by cervical decapitation. Blood was collected into heparinized tubes. Plasma was separated and used for the estimation of glucose, Vitamin C, and Vitamin E. Insulin was estimated by using radio immuno assay. The pancreatic tissues were excised, rinsed in ice-cold saline and homogenized in 0.1 M Tris—HCl buffer (pH 7.4).

**The Tissue Homogenate and Plasma Were Used for the Following Estimations** Lipid peroxidation was estimated using thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa et al. Lipid-hydroperoxides was assayed by the method of Jiang et al. Reduced glutathione was estimated by the method of Elman.

**Assay of Antioxidant Enzymes in Tissue Homogenate** Superoxide dismutase (SOD) was estimated using the method of Misra and Fridovich. Catalase (CAT) was assayed by the method of Takahara et al. Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. Total protein present in the tissue homogenate was estimated by the method of Lowry et al.

**Histopathological Studies** A portion of the pancreatic tissue was fixed in 10% buffered neutral formal saline for histological studies. After fixation, tissues were embedded in paraffin, solid sections were cut at 5 μm and stained with aldehyde. The sections were examined under light microscope and photomicrographs were taken.

**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.

**RESULTS**

Table 1 shows the levels of blood glucose and plasma insulin in normal and experimental groups of rats. The increased levels of blood glucose and decreased level of insulin in diabetic rats were restored to near normal levels in MC-seed extract and glibenclamide treated diabetic rats. Table 2 and Fig. 1 shows the levels of TBARS, lipid-hydroperoxides and reduced glutathione in plasma and pancreas of normal and experimental groups of rats. There was a significant increase in the levels of TBARS, lipid-hydroperoxides and a significant decrease in the level of reduced glu.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
<th>Plasma insulin (µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Normal control</td>
<td>81.76±5.81</td>
<td>85.25±4.96</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>263.91±18.42*</td>
<td>330.18±20.63*</td>
</tr>
<tr>
<td>Diabetic+MCSEt1</td>
<td>270.12±15.56*</td>
<td>93.52±4.34*</td>
</tr>
<tr>
<td>Diabetic+MCSEt2</td>
<td>270.41±17.11*</td>
<td>108.16±6.00*</td>
</tr>
<tr>
<td>Diabetic+glibenclamide</td>
<td>267.57±16.79*</td>
<td>121.32±6.09*</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D., for six rats per group. Values are statistically significant at *p<0.05; NS, non significant. Statistical significance was compared with the groups as follows: a) diabetic control rats were compared with normal control rats, b) MCSEt1 treated diabetic rats were compared with diabetic control rats, c) MCSEt2 treated diabetic rats were compared with diabetic control rats, d) glibenclamide treated diabetic rats were compared with diabetic control rats.
tathione in both plasma and pancreas of diabetic rats when compared to normal rats. Treatment with *Momordica charantia* seed extracts and glibenclamide significantly reversed these levels to near normal levels.

Figure 2 demonstrates the levels of vitamin C and vitamin E in plasma of normal and experimental groups of rats. The decreased level of vitamin C and increased level of vitamin E were observed during diabetes, when compared to normal control group. Treatment with *Momordica charantia* seed extracts and glibenclamide significantly reversed these levels to near normalcy.

Figure 3 shows the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in pancreas of normal and experimental groups of rats. A significant increase in the activities of SOD, CAT and GPx in pancreas was observed in diabetic rats when compared with normal rats. Treatment with *Momordica charantia* seed ex-

---

### Table 2. Effect of *Momordica charantia* on the Levels of Thiobarbituric Acid-Reactive Substances (TBARS), Lipid-Hydroperoxides and Reduced Glutathione in Plasma of Diabetic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (µmol/ml)</th>
<th>Lipid-hydroperoxides (10 nmol/dl)</th>
<th>Reduced glutathione (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.32±0.18</td>
<td>9.20±0.20</td>
<td>24.12±0.66</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>7.12±0.11*</td>
<td>14.00±0.17*</td>
<td>16.30±0.61*</td>
</tr>
<tr>
<td>Diabetic + MCSEt1</td>
<td>3.65±0.14*</td>
<td>10.08±0.26*</td>
<td>23.49±0.51*</td>
</tr>
<tr>
<td>Diabetic + MCSEt2</td>
<td>3.90±0.20*</td>
<td>10.80±0.25*</td>
<td>23.48±0.71*</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>4.24±0.22*</td>
<td>12.02±0.54*</td>
<td>31.50±0.14*</td>
</tr>
</tbody>
</table>

Values are given as mean±S.D., for six rats per group. Values are statistically significant at *p*<0.05; NS, non significant. Statistical significance was compared with in the groups as follows: a) diabetic control rats were compared with normal control rats, b) MCSEt1 treated diabetic rats were compared with diabetic control rats, c) MCSEt2 treated diabetic rats were compared with diabetic control rats, d) glibenclamide treated diabetic rats were compared with diabetic control rats.

---

**Fig. 1.** Effect of *Momordica charantia* on the Levels of Thiobarbituric Acid-Reactive Substances, Lipid-Hydroperoxides and Reduced Glutathione in Pancreas of Diabetic Rats

Values are given as mean±S.D., for six rats per group. Values are statistically significant at *p*<0.05; NS, non significant. Statistical significance was compared with in the groups as follows: a) diabetic control rats were compared with normal control rats, b) MCSEt1 treated diabetic rats were compared with diabetic control rats, c) MCSEt2 treated diabetic rats were compared with diabetic control rats, d) glibenclamide treated diabetic rats were compared with diabetic control rats.

---

**Fig. 2.** Effect of *Momordica charantia* on the Levels of Vitamin C and Vitamin E in Plasma of Diabetic Rats

Values are given as mean±S.D., for six rats per group. Values are statistically significant at *p*<0.05; NS, non significant. Statistical significance was compared with in the groups as follows: a) diabetic control rats were compared with normal control rats, b) MCSEt1 treated diabetic rats were compared with diabetic control rats, c) MCSEt2 treated diabetic rats were compared with diabetic control rats, d) glibenclamide treated diabetic rats were compared with diabetic control rats.
tricts and glibenclamide decreased the activities of SOD, CAT and GPx in diabetic groups of rats.

Figures 4a—e show the section of pancreas of control and experimental groups of rats. Fig. 4a presents the section of pancreas from normal control group showing normal islets. Diabetic pancreas showing atrophy of β-cells and vascular degenerative changes in the islets (Fig. 4b), MCSEt1 (Fig. 4c), MCSEt2 (Fig. 4d) and glibenclamide (Fig. 4e) treated diabetic pancreas showing increase in the islets as compared to diabetic treated.

DISCUSSION

The traditional medicine all over the world is now-a-days revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) are involved in a high number of diseases. Free radicals may play an important role in causation and complications of diabetes mellitus.27)

STZ is a valuable agent for experimental induction of type I diabetes. In type I diabetes, the insulin is markedly depleted but not absent28) It has been reported that STZ-diabetic animals may exhibit most of the diabetic complications mediated through oxidative stress, and the involvement of free radicals in pancreatic cell destruction.29) Keeping these in view, in the present study, the oxidative stress has been assessed in rats made diabetic by streptozotocin. Preliminary studies conducted by us revealed the non-toxic nature of MC seeds on normal rats. In the present study oral administration of MC seeds extract decreased the blood glucose level to near normal in diabetic rats. The possible mechanism by which extracts brings about decrease in blood glucose may be by stimulation of surviving β-cells of islets of langerhans to release more insulin. This was clearly evidenced by the increased level of plasma insulin in diabetic rats treated with MC seeds extract. The activation of β cells with bitter gourd seed treatment was reported in mildly STZ diabetic animals in which some β cells were found active and granulation returns to normal giving insulinogetic effect.29) In this context a number of other plants have also been reported to have antihyperglycemic and insulin stimulatory effects.30)

The sulfonylureas such as glibenclamide have been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β cells principally by inhibiting ATP-sensitive KATP channels in the plasma membrane.31) Courtois et al., have reported that oral administration of glibenclamide to the STZ-induced diabetic rats, decreased the blood glucose level and also could be considered as a standard antidiabetic drug to compare the efficacy of hypoglycemic compounds.32,33)

Almost all the major classes of biomolecules are attacked by free radicals but lipids are probably the most susceptible. Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs), which are readily attacked by oxidizing radicals. The oxidative destruction of PUFAs, known as lipid peroxidation, is particularly damaging because it proceeds as a self-perpetuating chain-reaction.34) Increased lipid peroxidation impairs membrane fluidity and changing the activity of membrane bound enzymes and receptors.35) The most popular and easiest method used to assay lipid peroxidation and free radical activity in biological sample is TBARS.36) A significant increase in TBARS and lipid-hydroperoxides in plasma and pancreas of diabetic rats suggest an increase in reactive oxygen species, that could be due to either their increased production or decreased destruction.37,38)

In the present study, the plasma and pancreas TBARS and lipid-hydroperoxides levels were significantly lowered in the extract treated group compared to diabetic control group. The protective effect of extract was also confirmed by the histopathological examination.

GSH has a multifaceted role in antioxidant defence. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases.39) Studies have shown that the plasma and pancreas GSH concentration of STZ-induced diabetic rats are significantly lowered when compared to normal rats.40) Decreased glutathione in diabetes could be the result of decreased synthesis or increased degradation of GSH by increased oxidative stress.38) Administration of Momordica charantia seed extracts and glibenclamide increased the content of GSH in plasma and pancreas of dia-
Vitamin C is one of the four dietary antioxidants, the other three being vitamin E, vitamin A precursor beta-carotene, and selenium. Frei reported that the ability of vitamin C to preserve the levels of other antioxidants in human plasma. Also, vitamin C regenerates vitamin E from its oxidized form. Hypoinsulinemia and/or hyperglycemia inhibit ascorbic acid and cellular transport. As the chemical structure of ascorbic acid is similar to that of glucose, it shares the membrane transport system with glucose and hence competes with it for its transport. We observed lowered levels of plasma vitamin C in diabetic rats. Thus, the elevation in glucose concentration may depress natural antioxidant like vitamin C or due to decrease in GSH levels, since GSH is required for recycling of vitamin C. The elevated levels of vitamin E in plasma may be due to increased intake of vitamin E per unit weight or increase in serum lipid levels or both in the diabetic rats.

The endogenous antioxidant enzymes (e.g., SOD, CAT and GPx) are responsible for the detoxification of deleterious oxygen radicals. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the disputation of superoxide radicals to produce $\text{H}_2\text{O}_2$ and molecular oxygen, hence diminishing the toxic effects caused by their radical. Catalase (CAT) is a hemoprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. The superoxide anion has been known to inactivate CAT, which is involved in the detoxification of hydrogen peroxide. GPx plays a primary role in minimizing oxidative damage. It has been proposed that GPx is responsible for the detoxification of $\text{H}_2\text{O}_2$ in low concentration whereas catalase comes into play when GPx pathway is reaching saturation with the substrate.

The present study shows that increased activity of SOD, CAT and GPx in diabetic rats, when compared to normal rats, which may be due the increase in the activity of these above enzymes and may result from radical induced activation. The obtained results are in agreement with earlier data. Significant increase in the activity of antioxidant enzymes in diabetic pancreas indicates an adaptive mechanism in response to oxidative stress.

In conclusion, the results of this study represent that administration of *Momordica charantia* seeds showed hypo-glycemic effect, which controls blood glucose level and thereby prevents the formation of free radicals or it may scavenge the reactive oxygen metabolites through various antioxidant compounds in them. Further studies are in progress to isolate the active components in *Momordica charantia* seeds and their role in controlling diabetes.

REFERENCES


Fig. 4. Histopathological Observations Made on the Pancreatic Tissue of control and Experimental Groups of Rats and the Photomicrographs Presented Are the Representatives of the Six Rats Used in Each Group (Aldehyde Fuschin, 320×)

(a) Presents the section of pancreas from normal control rat showing normal islets with clusters of purple stained $\beta$-cells. (b) Pancreas of STZ-induced diabetic rat showing atrophy of $\beta$-cells and vascular degenerative. A reduction in number and size of islets were observed. (c) MCSEt1, (d) MCSEt2 and (e) glibenclamide treated diabetic pancreas showing near normal architecture.