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Protective Role of *Momordica charantia* Seeds Extract on Membrane Bound ATPases and Lysosomal Hydrolases in Rats with Streptozotocin Diabetes

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Abstract: The aim of the present study was to examine the effect of an aqueous extract from *Momordica charantia* seed(s) on membrane bound ATPases and lysosomal hydrolases in the liver and kidney of streptozotocin (STZ)-induced diabetic rats. The rats treated with STZ showed a significant alterations in the activities of membrane bound ATPases and lysosomal hydrolases in the liver and kidney. Oral administration of *Momordica charantia* seed(s) extract at a dose of 150 mg kg^{-1} body weight rat^{-1} day^{-1} to STZ-induced diabetic rats for a period of 30 days significantly restored the alterations in enzymes activity to near normal level. These results were compared with glibenclamide, a reference drug. Thus, the present study confirms that *Momordica charantia* seed(s) extract possesses a significant protective effect on membrane bound ATPases and lysosomal hydrolases.

Key words: *Momordica charantia*, aqueous extract, streptozotocin-induced diabetes, membrane bound ATPases, lysosomal hydrolases

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system. Diabetes mellitus is recognized by chronic elevation of blood glucose level, which could results from a deficiency in β-cells of the endocrine pancreas and/or from a subsentivity to insulin in target cells (Jensen *et al*., 1988). In diabetes mellitus, chronic hyperglycemia produces multiple biochemical sequelae and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease (Giugliano *et al*., 1996). Several hypotheses have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products (AGEs) and enhanced glucose flux through the polyol pathway (Oberley, 1988). Elevated generation of free radicals resulting in the consumption of antioxidant defense components may lead to disruption of cellular functions and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation (Baynes, 1991). Therefore, crude drugs or natural diet food which possess both hypoglycemic and free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health (McCune and Johns, 2002). 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.

Among these herbal resources, two varieties of *Momordica charantia* seed were selected for the present study (i.e., MCSEt1 and MCSEt2). *Momordica charantia* (MC), commonly referred to as bittergourd or karela, belongs to the *Curcurbitaceae* family and is commonly consumed as a vegetable

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in India, as it is very cheap and available throughout the year. There are two varities of this vegetable based on the size and shape. The small variety is little, oval and dark green in colour (MCSEt1). The other one is large variety, long oblong and pale green in colour (MCSEt2). Different parts of this plant have been used in the Indian system of medicine for a number of ailments besides diabetes (Jayasooriya *et al*., 2000). Our previous experimental results revealed the glucose and lipid lowering properties of *Momordica charantia* seed(s) extract in streptozotocin (STZ)-induced diabetic rats (Sathishsekar *et al*., 2005; Sathishsekar and Subramanian, 2006).

The present study was aimed to evaluate the effect of an aqueous extract from the seeds of *Momordica charantia* (MCSEt1 and MCSEt2) on the activities of membrane bound ATPases and lysosomal hydrolases during STZ-induced diabetic rats. The results were compared with glibenclamide, a known hypoglycemic drug.

MATERIALS AND METHODS

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 and 1294).

Preparation of Seed(s) Extract

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 grounded 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 a known amount of 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, employed in folk medicine, was prepared freshly each time and administered orally. 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 Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No. 01/033/04).

Induction of Experimental Diabetes

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 sodium citrate buffer (pH 4.5) at a dose of 55 mg kg^{-1} body weight (Rakieten *et al*., 1963). The control rats received equivalent amount of sodium citrate buffer alone. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Hyperglycemia was confirmed one week after induction via blood glucose level measurement. STZ-treated animals were considered as diabetic when the fasting blood glucose levels observed were above 250 mg dL^{-1}.

Experimental Design

The animals were divided into five groups with six rats in each group.

Group I: Normal Control
Group II: Diabetic Control
Group III: Diabetic rats treated with MCSEt1 (150 mg kg^{-1} body weight $rat^{-1}day^{-1}$) in aqueous solution orally for 30 days.
Group IV: Diabetic rats treated with MCSEt2 (150 mg kg^{-1} body $weight^{-1}$ $rat^{-1}day^{-1}$) in aqueous solution orally for 30 days.
Group V: Diabetic rats administered with glibenclamide (600 μg kg^{-1} body weight $rat^{-1}day^{-1}$) in aqueous medium orally for 30 days.

Analytical Methods

After 30 days of experimental treatment, the 16 h fasted rats were sacrificed by cervical decapitation. The blood sample was collected with potassium oxalate and sodium fluoride solution for the estimation of glucose by O-toluidine method (Sasaki *et al*., 1972). Immediately after sacrifice, the liver and kidney were dissected out and immediately washed in ice-cold saline. Blotted with a filter paper, weighed and a portion of the tissues were homogenized in Tris- HCl buffer, pH 7.4 (0.1 M) with a Teflon homogenizer. The homogenate was used for the assays of Total ATPase by the method of Hokins *et al*. (1973), which was modified from the method of Evans (1969), Na^+ K^+ ATPase by the method of Bonting (1970), Ca^{2+} ATPase by the method of Hjerten and Pan (1983) and Mg^{2+} ATPase by the method of Ohnishi *et al*. (1982).

Lysosomal fraction was isolated by the method of Wattiaux (1977). The activity of lysosomal enzymes was assayed by the following methods. β-D-glucuronidase activity was measured using Hultberg *et al*. (1976) method. β-D-N-acetyl glucosaminidase activity was measured using Moore and Morris (1982) method. β-D-galactosidase by the method of Conchie *et al*. (1967). The activity of Cathepsin-D and Acid phosphatase was assayed according to the method of Sapolsky *et al*. (1973) and King (1965), respectively. Protein content in the tissue homogenate was measured by the method of Lowry *et el*. (1951).

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±SD for six animals in each group.

RESULTS

STZ-induced diabetic rats showed a significant increase in blood glucose level when compared with corresponding control rats. Following oral administration of MCSEt1, MCSEt 2 and glibenclamide, blood glucose level was found to be similar to those in control rats (Table 1).

There was a marked decrease in the activity of membrane bound ATPases such as Total ATPase, Na^+ K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase in the liver and kidney of STZ-induced diabetic rats when compared with activities in corresponding control rats. MCSEt1, MCSEt 2 and glibenclamide administration resulted in the normalization of these enzymes activity towards near normalcy (Table 2 and Fig. 1).

A significant elevation in the activity of liver lysosomal enzymes such as β-D-glucuronidase, β-D-N-acetyl glucosaminidase, β-D-galactosidase, Cathepsin-D and Acid phosphatase in STZ-induced diabetic rats was observed when compared with corresponding control rats. Administration of MCSEt1, MCSEt 2 and glibenclamide brought down the activity of these enzymes significantly in the liver of diabetic rats (Table 3).

Table 1: Changes in blood glucose level of control and experimental groups of rats

Groups	Blood glucose (mg dL^{-1})
Normal control	86.25±5.43
Diabetic control	307.36±23.36[a]
Diabetic+MCSEt 1	91.70±5.87[b]
Diabetic+MCSEt 2	102.40±6.55[b]
Diabetic+Glibenclamide	118.32±7.69[b]

Values are given as mean±SD for groups of six animals in each group; [a]p<0.05 when compared with control rats; [b]p<0.05 when compared with diabetic control rats

Table 2: Activities of membrane bound ATPases in the kidney of control and experimental groups of rats

Parameters	Normal control	Diabetic control	Diabetic + MCSEt 1	Diabetic + MCSEt 2	Diabetic + Glibenclamide
Total ATPase	6.95±0.43	3.31±0.25[a]	6.40±0.41[b]	6.07±0.40[b]	5.98±0.37[b]
Na^+K^+ ATPase	2.94±0.18	1.46±0.11[a]	2.87±0.18[b]	2.63±0.17[b]	2.60±0.16[b]
Mg^{2+} ATPase	2.81±0.17	1.57±0.12[a]	2.79±0.18[b]	2.65±0.16[b]	2.58±0.17[b]
Ca^{2+} ATPase	1.87±0.12	0.95±0.07[a]	1.78±0.11[b]	1.71±0.11[b]	1.69±0.10[b]

Values are given as mean±SD for groups of six animals in each group; [a]p<0.05 when compared with control rats; [b]p<0.05 when compared with diabetic control rats; Activity was expressed as: μmoles of phosphate liberated min^{-1} mg^{-1} protein

Table 3: Activities of lysosomal hydrolases in the liver of control and experimental groups of rats

Parameters	Normal control	Diabetic control	Diabetic + MCSEt1	Diabetic + MCSEt 2	Diabetic + Glibenclamide
β-D-glucuronidase	21.02±1.26	36.12±2.75[a]	22.71±1.43[b]	22.94±1.49[b]	23.01±1.50[b]
β-D-N-acetyl glucosaminidase	48.83±3.08	63.78±4.84[a]	48.17±3.00[b]	53.94±3.40[b]	53.62±3.43[b]
β-D-galactosidase	39.17±2.47	50.08±3.81[a]	41.16±2.63[b]	42.80±2.78[b]	46.22±2.96[b]
Cathepsin-D	19.53±1.21	32.15±2.48[a]	21.87±1.40[b]	22.94±1.45[b]	23.07±1.52[b]
Acid phosphatase	108.13±6.82	158.76±12.07[a]	120.25±7.70[b]	130.98±8.51[b]	131.04±8.39[b]

Values are given as mean±SD for groups of six animals in each group; [a]p<0.05 when compared with control rats; [b]p<0.05 when compared with diabetic control rats; Activity was expressed as: μmoles of p-nitrophenol liberated h^{-1} mg^{-1} protein for β-D Glucuronidase; β-D-N-Acetyl glucosaminidase and β-D-Galactosidase; μmoles of tyrosine released h^{-1} mg^{-1} protein for Cathepsin-D; μmoles of phenol liberated h^{-1} 100 mg protein for Acid phosphatase

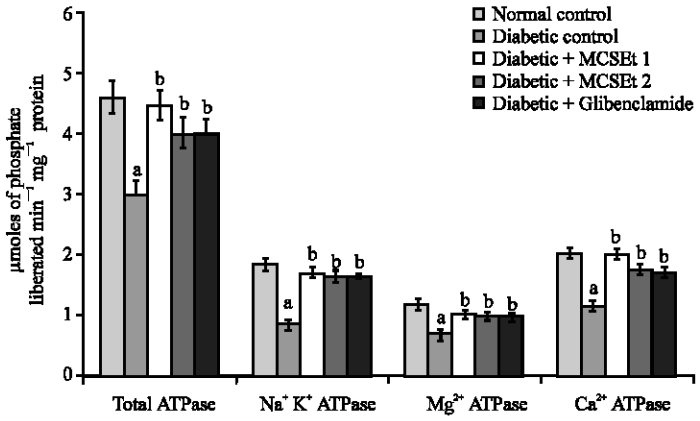

Fig. 1: Activities of membrane bound ATPases in the liver of control and experimental groups of rats Values are given as mean±SD for groups of six animals in each group; [a]p<0.05 when compared with control rats; [b]p<0.05 when compared with diabetic control rats

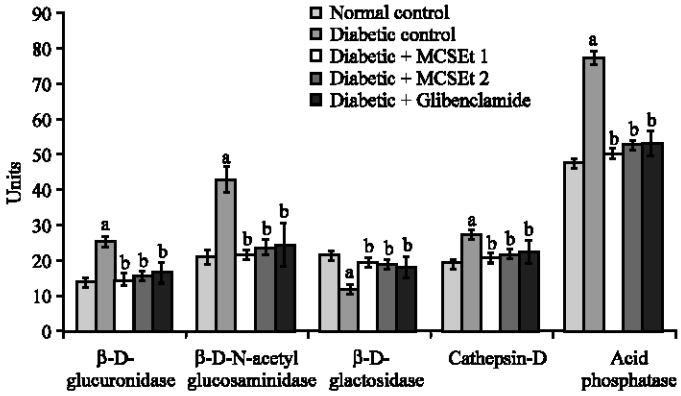

Fig. 2: Activities of lysosoml hydrolases in the kidney of control and experimental groups of rats Values are given as mean±SD for groups of six animals in each group; $^{a}p<0.05$ when compared with control rats; $^{b}p<0.05$ when compared with diabetic control rats; Activity was expressed as: μmoles of p-nitrophenol liberated h^{-1} mg^{-1} protein for β-D Glucuronidase; β-D-N-Acetyl glucosaminidase and β-D-Galactosidase; μmoles of tyrosine released h^{-1} mg^{-1} protein for Cathepsin-D; μmoles of phenol liberated h^{-1} 100 mg protein for acid phosphatase

A significant increase in the activity of β-D-glucuronidase, β-D-N-acetyl glucosaminidase, Acid phosphatase and Cathepsin-D in the kidney of STZ-induced diabetic rats was found (Fig. 2). In contrast, there was a significant decrease in the activity of β-D-galactosidase was observed in the kidney of diabetic rats. However, following treatment with either MCSEt1 or MCSEt 2 or glibenclamide, the activity of these enzymes was brought back to near normalcy.

DISCUSSION

Diabetes mellitus is known to promote deterioration of membrane function and impair intra cellular metabolism in the organism (Parinandi *et al*., 1990). This study was there fore under taken to assess the effect of *Momordica charantia* seed(s) extract on membrane bound ATPases and lysosomal hydrolases in STZ-induced diabetic rats. Membrane bound ATPases are ubiquitous enzymes essential for the maintenance of electrolyte balance and fundamentally involved in the maintenance of ion gradients that drive the co-transport of amino acids and sugars, regulate cell volume and contribute to part of the membrane potential (Sweeney and Klip, 1998). Alterations of these transport enzymes are thought to be linked to several diabetic complications, for example hypertension (Shahid and Mahboob, 2003). Alterations in membrane bound ATPases activity have been observed in various tissues. STZ-induced diabetes is also characterized by a severe derangement of subcellular metabolism and structural alterations of cell membranes (Brasitus and Dudeja, 1985).

The plasma membrane Na^+K^+ ATPase is concerned with the maintenance of a low concentration of Na^+ and consequently of cellular water content. Lowered activity of Na^+ K^+ ATPase can lead to a decrease in sodium efflux and thereby alter the membrane permeability (Finoth and Palatini, 1986). A decrease in the activity of Na^+ K^+ ATPase in diabetes would be expected in light of the many reports that insulin stimulates this enzyme and sodium pump activity *in vivo* and *in vitro* in a variety of tissues. Further, Na^+ K^+ ATPases are lipid-dependent as well as SH-dependent membrane-bound enzymes, oxidation of thiol groups by Reactive Oxygen Species (ROS) is reported to inhibit the enzyme activity in diabetic condition (Thomas and Reed, 1990). STZ-induced diabetic rats are well

known to have high blood glucose concentration. Much evidence has indicated that some biochemical pathways strictly associated with hyperglycemia (non-enzymatic glycosylation, glucose autoxidation and polyol pathways) can increase the production of free radicals (Baynes, 1991). Thus, the oxygen-derived species that formed during diabetes mediate the cell membrane damage forming lipid hydroperoxides and ultimately leading to the loss of functional integrity of the membrane and this might be a reason for the observed decrease in Na^+ K^+ ATPase activity in the present study. Ca^{2+} ATPase regulates the calcium pump activity (Levy *et al*., 1994). Decrease in the activity of Ca^{2+} ATPase can increase intracellular concentration of Ca^{2+} and alter the signal transduction pathways and cellular fluidity (Aubier and Viires, 1998). Glycosylation of membrane proteins is reported to inhibit Ca^{2+} ATPase activity significantly and the rate of glycosylation depends on the proportion of sugar present in carbonyl form (Ramanadevi *et al*., 1997). The activity of Ca^{2+} ATPase is also modulated by cellular thiol status (degree to thiol oxidation and lipid peroxidation) (Bironaite and Ollinger, 1997). Mg^{2+} ATPase activity is involved in other energy-requiring process in the cell and its activity is sensitive to membrane lipid peroxidation. STZ-induced diabetes was shown to have decreased activity of Mg^{2+} ATPase (Levy *et al*., 1994). In general, lipid peroxidation and increased rate of glycosylation can inturn diminish the activity of different ATPases in diabetic condition. *In vivo* insulin treatment restoring all the altered membrane ATPases activity to near normal (Jourdheuil *et al*., 1987) and also insulin is responsible for the regulation of membrane ATPases activities (Sweeney and Klip, 1998). In the present study, oral administration of MC seed(s) extract for 30 days significantly reduced the diabetes-induced alterations in the activities of membrane-bound ATPases, possibly by scavenging of free radicals and there by decreasing the lipid peroxidative damage to the cell membranes (Sathishsekar and Subramanian, 2005a, 2005b) and/or by its hypoglycemic effect.

Lysosomes are distinct group of cytoplasmic organelles, known to occur in numerous animal tissues and characterized by their content of a variety of hydrolytic enzymes. Degradation of connective tissue constituents such as collagen and glycoproteins are brought about by enzymes primarily by lysosomal origin. An increased activity of liver lysosomal enzymes (Geetha, 1993; Olsen *et al*., 1990; Kelly and Woodward, 1988) was observed in diabetic rats. This may be attributed towards persistent hyperglycemia, which leads to alterations in sugar moiety of glycoproteins, excess glycation of proteins, excess catabolism of macromolecules and excess free radical production (Witek *et al*., 2001). A significant increase in the activities of β-D-Glucuronidase, β-D-N-Acetyl glucosaminidase, Acid phosphatase, Cathepsin-D and a concomitant decrease in the activity of β-D-galactosidase was observed in the kidney of STZ-induced diabetic rats. This is in agreement with those reported earlier (Belfiore *et al*., 1986; Nerurkar *et al*., 1988). Osicka *et al*. (2001) suggested that diabetes induced changes in renal lysosomal processing is one of the initial events in the development of diabetic nephropathy. The β-D-N-acetyl glucosaminidase has been regarded as a possible marker of renal damage. It has been suggested that the β-D- N-acetyl glucosaminidase reflects lysosomal dysfunction of both glomerular and proximal tubular epithelial cells, which may be the cause for poor glycemic control, its reflect brush border damage of proximal tubules which may be caused by diabetic nephropathy (Fushimi and Tarui, 1974). Belifiore *et al*. (1986) have observed a decrease in the activity of β-D-galactosidase in kidney of diabetic mice, which lead to the thickening of basement membrane and the development of microangiopathy. Thus, the decreased β-D-galactosidase activity in diabetic condition is due to the increased formation of hydroxyl lysine-linked α-glucosyl-β-galactose disaccharide unit in renal glomerular basement membrane (Beisswenger and Spiro, 1970). Administration of MC seed(s) extract to diabetic rats significantly restores the activity of lysosomal enzymes which may be attributed to its hypoglycemic effect.

In conclusion, diabetes induces disturbances in the activities of membrane bound phosphatases and lysosomal hydrolases. This study demonstrates that in STZ-induced diabetic model, *Momordica charantia* seed(s) extract administration ameliorated the changes in the activities of

membrane bound ATPases and lysosomal hydrolases. The mechanism by which *Momordica charantia* seed(s) extract improves these alterations in diabetic rats is probably by its hypoglycemic and free radical scavenging properties. There is an on going research to isolate and characterize the bioactive compound(s) responsible for the antidiabetic/antioxidative action in these crude extract and to use the (se) compound(s) in a bioassay-directed experiment.

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