

## Original Article

**Antioxidant properties of *Momordica Charantia* (bitter gourd) seeds on Streptozotocin induced diabetic rats**

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The aim of the present study is to investigate the antioxidant activities of the aqueous extract of seeds of two varieties, namely a country and hybrid variety of *Momordica charantia* (MCSEt1 and MCSEt2) respectively in streptozotocin induced diabetic rats. Oral administration of both the seed extracts at a concentration of 150 mg/kg b.w for 30 days showed a significant decrease in fasting blood glucose, hepatic and renal thiobarbituric acid reactive substances and hydroperoxides. The treatment also resulted in a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase in the liver and kidney of diabetic rats. The results clearly suggest that seeds of *Momordica charantia* treated group may effectively normalize the impaired antioxidant status in streptozotocin induced-diabetes than the glibenclamide treated groups. The extract exerted rapid protective effects against lipid peroxidation by scavenging of free radicals there by reducing the risk of diabetic complications. The effect was more pronounced in MCSEt1 compared to MCSEt2.

**Key Words:** *Momordica charantia*, bitter gourd seeds, streptozotocin, diabetes, antioxidant enzymes.**Introduction**

Diabetes mellitus is probably the fastest growing metabolic disorder in the world and it is a major source of morbidity in developed countries. Once regarded as a single disease entity, diabetes is now regarded as a heterogeneous group of diseases characterized by a state of chronic hyperglycemia, which causes a number of secondary complications like cardiovascular, renal, neurological and ocular.<sup>1</sup> There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals.<sup>2</sup> A free radical is any species capable of independent existence that contains one or more unpaired electrons. Thus, free radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation. Increased generation of reactive oxygen species (ROS) and lipid peroxidation has been found to be involved in the pathogenesis of many diseases of known and unknown etiology and in the toxic actions of many compounds.<sup>3</sup> Antioxidants thus play an important role to protect the human body against damage caused by reactive oxygen species.<sup>4</sup> The endogenous antioxidant enzymes (e.g SOD, CAT, GSH and GPx) are responsible for the detoxification of deleterious oxygen radicals.<sup>5</sup>

In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defenses.<sup>6</sup> Hence, compounds with both hypoglycemic and antioxidative properties would be useful antidiabetic agents.<sup>7</sup> Many plant extracts and plant products have been shown to have significant antioxidant activity,<sup>8</sup> which may be an important property of plant medicines associated with the treatment of several ill fated diseases including diabetes. Thus, herbal plants are considered useful means to prevent and/or ameliorate certain disorders,

such as diabetes, atherosclerosis and other complications.<sup>9</sup> Among these herbal resources, *Momordica charantia* seed varieties are selected for the present study.

*Momordica charantia* (MC), commonly referred to as bittergourd or Karela, belongs to the cucurbitaceae family and is commonly consumed as a vegetable in India, as it is very cheap and available throughout the year. Our previous experimental results were highly encouraging as they revealed that blood glucose level was significantly lower after oral administration of aqueous extract of *Momordica charantia* seeds in glucose load condition and in streptozotocin – induced diabetes.<sup>10</sup> Different parts of this plant have been used in the Indian system of medicine for a number of ailments besides diabetes.<sup>11,12</sup> The present investigation was undertaken to assess the effect of an aqueous extract from the seeds of *Momordica charantia* (MCSEt1 and MCSEt2) on tissue lipid peroxides and enzymatic antioxidant in streptozotocin (STZ) induced diabetic rats.

**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.

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### 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 grounded into fine powder by an electrical mill and mesh (mesh number 50). The powdered seeds were kept in air-tight 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, 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 gms 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 (Approval number: 360/01/A/CBCSEA).

### Induction of diabetes

STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents.<sup>13,14</sup> After overnight fasting (deprived of food for 16 hours had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ (Sigma, St. Louis, Mo) dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 55mg/kg body weight.<sup>15</sup> The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg /dl) were considered as diabetic rats and used for the further experiments. The change in the body weight was observed throughout the treatment period in the experimental animals.

### Experimental set up

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

Group I: Normal control

Group II: Diabetic control

Group III: Diabetic rats treated with MCSEt1 (150mg/kg b.w/day) in aqueous solution orally for 30 days.

Group IV: Diabetic rats treated with MCSEt2 (150mg/kg b.w/day) in aqueous solution orally for 30 days.

Group V: Diabetic rats administered with glibenclamide (600µg/kg b.w/day) in aqueous solution orally for 30 days<sup>16</sup>

After 30 days of treatment the fasted rats were sacrificed by cervical decapitation and the blood was collected using potassium oxalate and sodium fluoride as anticoagulant for estimation of fasting blood glucose. The liver and kidneys were dissected out, washed in ice-cold saline, patted dry and weighed.

### Biochemical Assays

Fasting blood glucose was estimated by O-toluidine method.<sup>17</sup> Thiobarbituric acid reactive substances (TBARS) were estimated by the method of Okhawa *et al.*<sup>18</sup> Hydroperoxide was determined by the method of Jiang *et al.*,<sup>19</sup> reduced glutathione (GSH) was estimated by the method of Sedlak *et al.*<sup>20</sup> The antioxidant enzymes were assayed by the following methods. Superoxide dismutase (SOD) by the method of Misra *et al.*,<sup>21</sup> catalase (CAT) by the method of Takahara *et al.*,<sup>22</sup> glutathione peroxidase (GPx) and glutathione-S-transferase (GST) by Rotruck *et al.*,<sup>23</sup> Habig *et al.*,<sup>24</sup> protein content in tissue homogenate was measured by the method of Lowry *et al.*,<sup>25</sup> respectively.

### Statistical analysis

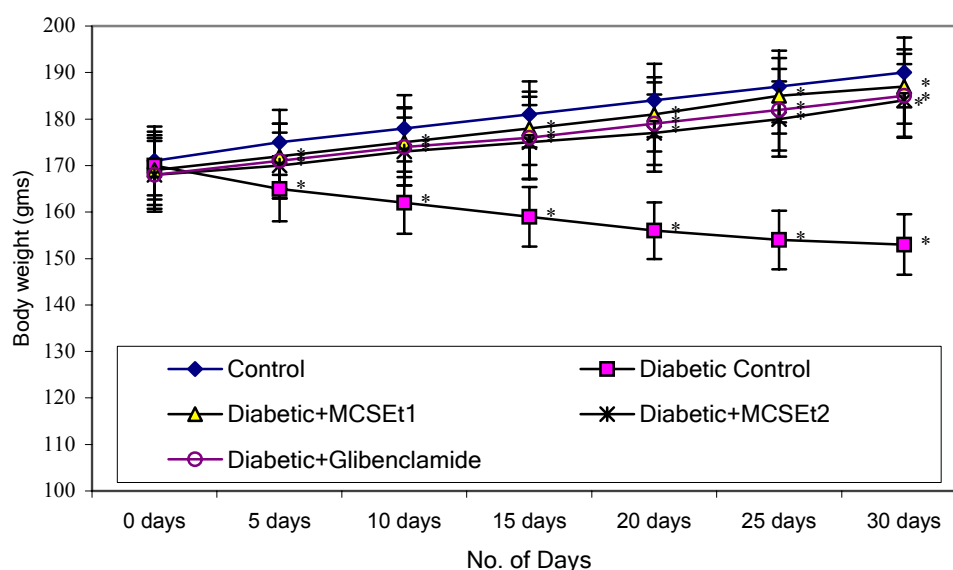
All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All the results were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

### Results

Figure 1 shows the body weight of control and experimental groups of rats. A significant decrease in body weight was observed in streptozotocin induced diabetic rats (Group II) when compared to the control group of rats (Group I). *Momordica charantia* extracts and glibenclamide administered rats (Group III, IV and V) showed progressive increase in body weight.

Table 1 shows the fasting blood glucose and urine glucose levels in control and experimental groups of rats. There was a significant increase in blood glucose and glycosuria in diabetic control group compared to normal control rats. Administration of *Momordica charantia* extract (MCSEt1 and MCSEt2) and glibenclamide tends to bring down the blood glucose concentrations compared to untreated diabetic rats and totally controlled glycosuria. The effect was trend in the groups of rats administered with *Momordica charantia* extract 1 (MCSEt1).

Table 2 and 3 shows the concentration of TBARS, hydroperoxides and reduced glutathione in liver and kidney of control and experimental groups of rats. The levels of TBARS and hydroperoxides in diabetic rats were significantly higher than control rats, whereas diabetic rats treated with the aqueous extracts (MCSEt1 and MCSEt2) and glibenclamide restored the altered values to the near normalcy. The decreased concentration of GSH was observed in diabetic rats. Administration of aqueous extracts (MCSEt1 and MCSEt2) and glibenclamide tends to bring the GSH level to near normal. The effect was more pronounced in the groups of rats administered with *Momordica charantia* extract 1 (MCSEt1). Tables 4 and 5 show the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in the



**Figure 1.** Changes in body weight of control and experimental groups of rats. Values are given as mean  $\pm$  SD for groups of six animals each. Values are statistically significant at  $*P < 0.05$ . Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.

**Table 1.** Levels of blood glucose and urine glucose in control and experimental groups of rats

Groups	Blood glucose (mg/dl)	Urine glucose
Normal Control	87.12 $\pm$ 3.00	Nil
Diabetic Control	275.18 $\pm$ 12.50*	+
Diabetic + MCSEt1	92.18 $\pm$ 5.00*	Nil
Diabetic + MCSEt2	95.15 $\pm$ 5.16*	Nil
Diabetic + Glibenclamide	114.48 $\pm$ 12.08*	Nil

Values are given as mean  $\pm$  SD for groups of six animals each. Values are statistically significant at  $*P < 0.05$ ; Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control. (+) Indicates more than 2% sugar.

liver and kidney of control and experimental groups of rats. The activities of SOD, CAT, GPx and GST in tissues, such as liver and kidney was significantly lower in diabetic control rats compared to diabetic rats treated with *Momordica charantia* extracts and glibenclamide. The effect was more pronounced in the diabetic rats treated with *Momordica charantia* extract 1 (MCSEt1).

## Discussion

The present study was conducted to evaluate the beneficial effects of two varieties of bittergourd (*Momordica charantia*) seed extracts on antioxidant status in STZ induced diabetic rats. The preliminary studies conducted by us revealed the non-toxic nature of MCseeds on normal rats.

STZ – induced experimental diabetes is a valuable model for type I diabetes. It has been stated that STZ diabetic animals may exhibit most of the diabetic complications mediated through oxidative stress.<sup>26</sup> Studies also suggest free

**Table 2.** Levels of lipid peroxides, hydroperoxides and reduced glutathione in liver of control and experimental groups of rats

Groups	TBARS (mM/100g of tissue)	Hydroperoxides (mM/100g of tissue)	Glutathione (GSH) (mg/100 g of tissue)
Normal Control	0.82 $\pm$ 0.02	68.02 $\pm$ 0.95	49.52 $\pm$ 1.23
Diabetic Control	1.92 $\pm$ 0.18*	98.02 $\pm$ 1.18*	23.52 $\pm$ 1.48*
Diabetic + MCSEt1	0.93 $\pm$ 0.05*	75.02 $\pm$ 2.05*	45.50 $\pm$ 3.66*
Diabetic + MCSEt2	1.08 $\pm$ 0.23*	81.00 $\pm$ 1.02*	42.72 $\pm$ 3.90*
Diabetic + Glibenclamide	1.52 $\pm$ 0.32*	85.05 $\pm$ 0.50*	39.02 $\pm$ 1.32*

Values are given as mean  $\pm$  SD for groups of six animals each. Values are statistically significant at  $*P < 0.05$ . Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.

radical involvement in pancreatic cell destruction.<sup>27</sup> Glibenclamide is often used as an insulin stimulant in many studies and also used as a standard antidiabetic drug in STZ- induced moderate diabetes to compare the antidiabetic properties of a variety of hypoglycemic compounds.<sup>28</sup>

STZ induced diabetes is characterized by severe loss in body weight<sup>29</sup> and this was also seen in the present study. *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide administration controlled this loss in body weight. However, it did not normalize the body weight completely as it remained lesser than normal control rats. The decrease in body weight observed in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source.<sup>30</sup>

**Table 3** Levels of lipid peroxides, hydroperoxides and reduced glutathione in kidney of control and experimental groups of rats.

Groups	TBARS (mM/100 g of tissue)	Hydro peroxides (mM/100 g of tissue)	Glutathione (GSH) (mg/100 g of tissue)
Normal Control	1.33 ± 0.07	55.53 ± 0.76	36.33 ± 2.03
Diabetic Control	2.23 ± 0.05*	77.02 ± 1.08*	24.63 ± 1.37*
Diabetic + MCSEt1	1.48 ± 0.02*	64.78 ± 0.87*	33.51 ± 1.50*
Diabetic + MCSEt2	1.64 ± 0.01*	68.64 ± 0.90*	31.43 ± 1.27*
Diabetic + Glibenclamide	1.84 ± 0.05*	70.75 ± 0.90*	28.94 ± 1.48*

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at \* $P < 0.05$ . Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.

**Table 4.** Activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase in liver of control and experimental groups of rats

Groups	SOD	CAT	GPx	GST
Normal Control	.48 ± 0.39	82.50 ± 3.02	9.96 ± 0.52	7.21 ± 0.75
Diabetic Control	3.92 ± 0.18*	40.32 ± 2.96*	5.52 ± 0.25*	3.42 ± 0.20*
Diabetic + MCSEt1	6.52 ± 0.14*	70.43 ± 3.63*	8.64 ± 0.43*	6.32 ± 0.18*
Diabetic + MCSEt2	5.89 ± 0.25*	69.72 ± 3.12*	8.02 ± 0.48*	5.92 ± 0.25*
Diabetic + Glibenclamide	5.18 ± 0.25*	67.25 ± 2.16*	7.85 ± 0.21*	5.54 ± 0.32*

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at \* $P < 0.05$ ; Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control. Activity is expressed as: 50% of inhibition of epinephrine auto oxidation per min for SOD; μmoles of hydrogen peroxide decomposed per min per mg of protein for catalase; μmoles of glutathione oxidized per min per mg of protein for GPx; units per min per mg of protein for GST.

The treated groups increase glucose metabolism and thus enhance body weight in STZ-induced diabetic rats to certain extent. The possible mechanism by which MCseeds brings about decrease in blood glucose and urine sugar may be by potentiation of the insulin effect by increasing either the pancreatic secretion of insulin from β cells of islets of Langerhans or its responsiveness.<sup>31</sup>

Hyperglycemia results in free radical formation through various biochemical reactions. Free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radical produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn results in elevated production of free radicals.<sup>32</sup> Lipid peroxide mediated tissue damage has been observed in the development of both type I and type II diabetes. It has been observed that insulin secretion is closely associated with lipoxygenase-derived peroxides.<sup>33</sup>

The increased lipid peroxidation in the diabetic animals may be due to the observed remarkable increase in the concentration of TBARS and hydroperoxides (lipid peroxidative markers) in the liver and kidney of diabetic rats.<sup>34</sup>

Nakakimura and Mizuno have reported that the concentration of lipid peroxides increases in the kidney of diabetic rats.<sup>35</sup> In the present study, TBARS and hydroperoxides levels in liver and kidney were significantly lower in the extract – treated group compared to the diabetic control group. The above result suggests that the aqueous extract may exert antioxidant activities and protect the tissues from lipid peroxidation.

GSH has a multifactorial role in antioxidant defense. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases.<sup>36</sup> Loven *et al.*, suggested that the decrease in tissue GSH could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes.<sup>37</sup> Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased hepatic GSH content. In the present study, the elevation of GSH levels in liver and kidney was observed in the *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide treated diabetic rats. This indicates that the *Momordica charantia* extracts (MCSEt1 and MCSEt2) and glibenclamide can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects.

SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H<sub>2</sub>O<sub>2</sub> and molecular oxygen,<sup>38</sup> hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H<sub>2</sub>O<sub>2</sub> or by glycation of enzymes.<sup>39</sup> The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide.<sup>40</sup> Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase.

Catalase (CAT) is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals.<sup>41</sup> This decrease in CAT activity could result from inactivation by glycation of enzyme.<sup>42</sup> Reduced activities SOD and CAT in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides.<sup>43</sup> The reductions of hepatic SOD and CAT activities in STZ - induced diabetic rats when compared with normal rats were reported.<sup>44</sup> Whereas, the extract treated groups showed a significant increase in the hepatic SOD and CAT activities of the diabetic rats. This means that the extracts can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes.

GPx plays a primary role in minimizing oxidative damage. Glutathione peroxidase (GPx), an enzyme with selenium, and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of H<sub>2</sub>O<sub>2</sub> or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione.<sup>45</sup> Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme.<sup>46</sup> Reduced activity of GST observed in the diabetic state may be due to the inactivation caused by reactive oxygen species.<sup>3</sup> Glutathione is a substrate of these two

enzymes and increased levels of glutathione in turn enhances the activity of GPx and GST and thus GPx and GST activity were induced to scavenge free radicals in diabetic rats.

Reduced activities of GPx and GST in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of toxic products. In this context, other workers also reported a decrease in the activity of these antioxidant enzymes (SOD, CAT, GPx and GST) in the liver and kidneys of diabetic rats.<sup>47</sup> Administration of *Momordica charantia* extracts and glibenclamide increased the activities of GPx and GST in the tissues of diabetic rats.

In conclusion, the present study showed that *Momordica charantia* seeds possess potent antioxidant activity, which may be directly or indirectly responsible for its hypoglycemic property. Further studies are in progress to identify the active components in *Momordica charantia* and their role in controlling diabetes.

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