**Momordica charantia** (Cucurbitaceae) methanolic extract alleviates alloxan-induced oxidative stress and β-cell damage in rat pancreas

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**ABSTRACT**

The aim of the present study was to evaluate the possible pancreatic protective effects of *Momordica charantia* methanolic extract (MCE) against pancreas β-cells’ damage and antioxidant defense systems in alloxan induced diabetes rats. Experimental diabetes was induced by a single dose of alloxan (150 mg/kg) administered by intraperitoneal way. The oxidative stress was measured by tissue MDA levels, reduced glutathione (GSH) content and by enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in pancreas. Biochemical observations were further substantiated with histological examination of pancreas. The increase in blood glucose and MDA levels with the decrease in GSH content and in enzymatic activities were the salient features observed in diabetic rats. Administration of MCE (300 mg/kg bw/day, orally) for 30 days caused a significant reduction in blood glucose and MDA levels in alloxan treated rats when compared with diabetic rats. Furthermore, diabetic rats treated with MCE showed a significant increase in the activities of both enzymatic and non-enzymatic antioxidants when compared to those of diabetic rats. Degenerative changes of pancreatic β-cells in alloxan treated rats were minimized to near normal morphology by administration of MCE as evidenced by histopathological examination.

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**KEYWORDS**

*Momordica charantia*; Diabetes mellitus; Oxidative stress; Antidiabetic; Antioxidant.

**INTRODUCTION**

The increasing incidence of diabetes represents an enormous socio-economic burden in the developing countries. The World Heath Organization estimates that over 300 million people worldwide will have (Diabetes mellitus) DM by the year 2025 with alarming proportions from developing countries[1].

DM is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secrete insulin. There are two main forms of diabetes. Type 1 diabetes is due primarily to autoimmune-mediated destruction of pancreatic islet beta-cells, resulting in dramatic insulin defi-
Momordica charantia (Cucurbitaceae) methanolic extract alleviates hyperglycemia. Its frequency (~ 10%) is low relative to Type 2 diabetes (T2D), which accounts for over 90% of cases. T2D is characterized by abnormal insulin secretion, associated with varying degrees of insulin resistance\(^2\).

β cells normally compensate insulin resistance by secreting more amounts of insulin to maintain the glucose homeostasis. In the course of time, however, this beta cell function gets impaired leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and frank diabetes. There occurs only a relative insulin deficiency as the day-long circulating insulin concentrations in diabetic patients that are almost comparable or slightly elevated in absolute terms to the values in normal individuals\(^3\).

β cell dysfunction results from prolonged exposure to high glucose, ROS or a combination of both. β-cells are particularly sensitive to ROS because they are low in free-radical quenching (antioxidant) enzymes such as (CAT), (GPx) and SOD. Therefore, the ability of oxidative stress to damage mitochondria and (markedly blunt insulin secretion is not surprising\(^4\).

As a consequence, blood glucose levels rise, passing from normal to impaired glucose tolerance first, and to overt diabetes eventually. Notably, deterioration of diabetes control and insulin secretory function occurs with years in diabetic patients despite insulin resistance remains stable. Thus, β-cell dysfunction is central to the development of diabetes, possibly due to a combination of decreased beta-cell mass and insulin secretion defects\(^5\).

Alloxan, a β-cytotoxin, has demonstrated severe physiological and biochemical derangements of the diabetic state. The alloxan rats exhibited severe glucose intolerance and metabolic stress as well as hyperglycemia due to a progressive oxidative insult interrelated with a decrease in endogenous insulin secretion and release\(^6\).

Momordica charantia (MC, family Cucurbitaceae), commonly known as bitter melon (BM). The seeds, fruit, leaves, and root of the plant have been used in traditional medicine for microbial infections, inflammation, hypertension, and as a laxative and emetic. Clinical conditions for which MC fruit extracts are currently being used in treatment of diabetes and dyslipidemia\(^7\).

The seeds, fruit, leaves, and root of the plant have been used in traditional medicine for microbial infections, sluggish digestion and intestinal gas, menstrual stimulation, wound healing, inflammation, fever reduction, hypertension, and as a laxative and emetic. Clinical conditions for which MC fruit extracts are currently being used in treatment of diabetes, dyslipidemia, microbial infections, and potentially as a cytoprotective agent for certain types of cancer. Although they have not been definitively determined.

Methanolic extract of Momordica charantia (MCE) contain lectin, insulin-like peptide (plant (p)-insulin), Oleanolic acidglycoside that delay intestinal sugar absorption, Citrulline that increase the half life of NO in blood vessels and vitamin C that scavenger ROS as shown in Figure 1\(^8\).

The present investigation is carried out in order to study the possible antihyperglycemic and antioxidant...
effects of MCE in order to attenuate pancreatic damage in alloxan-diabetic rats.

MATERIALS AND METHODS

Chemicals

Alloxan monohydrate was purchased from sigma Fine chemicals. All other chemicals used for this study were of analytical grade and obtained from Stanbio Laboratory USA Kits. Kits for the estimation of total cholesterol, triglyceride and HDL-cholesterol were purchased from Diamond Diagnostic Egypt.

Plant material

The fruits were collected from wildly growing populations on El-Ketar mountain, Hurghada, Egypt were authenticated by Dr. Hany Ezzat Khalil (Pharmacognacy Department, Faculty of Pharmacy, El Minia, Egypt.

Preparation of plant extract

The freshly whole ripe fruits (purchased from local suppliers) collected and washed with distilled water and air-dried under the control conditions and powdered. The powdered plant material was percolated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 80% methanol for 3 days. The extract was filtered, concentrated on rotavapour and then freeze-dried under high vacuum (1.3 Pa) and at temperature of -40 ± 2. The extract will be dissolved in 0.5 g Carboxy methyl cellulose (0.5w/v) ° for oral administration. Oral dose: 300 mg/kg BW of rats daily in accordance with[9].

Animals

30 Healthy male albino rats (5-7 months old, weighing 190-210 g) were procured from Faculty of Agricultural, El Minia University, Egypt. They were housed under standard laboratory conditions of light (12:12 h L: D cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). The animals were provided standard rat pellet feed and tap water ad libitum. Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Egypt.

Induction of experimental diabetes

For the present study, animals were divided into following 3 groups:

- **NC** (normal control), **DC** (diabetic control) and **MCE** (diabetic + Momordica charantia methanolic extract treated). After fasting for 18 hours, rats of group DC, MCE were made diabetic by a single intra-peritoneal injection of alloxan monohydrate, 150 mg/kg body wt, freshly dissolved in normal saline[10]. Subsequent to alloxan administration the rats had free access to food and water and were provided with 50% glucose solution to drink overnight to counter drug induced hypoglycemic shock. One week after alloxan injection, the fasting blood glucose (FBG) concentration was determined by means of one touch ultra glucometer (Johnson & Johnson Company, USA) and compatible blood glucose strips[11]. Rats showing fasting blood level greater than 140 mg/dl were considered diabetic and selected for treatment with methanolic extract of Momordica charantia (MCE) (300mg/kg body wt.). The MCE were administered orally, once in a day for 30 days.

Experimental design

The rats were randomly divided into four groups of seven animals each:

- **Group I (NC)**: normal control rats, received vehicle solution (1 ml/kg, intraperitoneal way) for 30 days.
- **Group II (DC)**: diabetic control rats, received alloxan in single dose (150 mg/kg bw, intraperitoneal way).
- **Group III (DC + MCE)**: MCE-treated diabetic rats received by oral way, 3 days after alloxan treatment, 300mg /kg, bw of MCE extract for 30 days.

On the last day of experiment, animals were sacrificed and blood samples were collected without heparin for biochemical estimations. Some pancreas were removed, cleaned and washed in ice-cold normal saline solution for biochemical analysis. Other portions of pancreas were taken out, washed in ice-cold saline solution and immediately fixed in 10% neutral buffered formalin solution.

Biochemical assays

**Biochemical estimations in blood and serum insulin**

Fasting blood glucose (FBG) concentration of all the four experimental groups was determined by
TABLE 1: Body weight, serum glucose and insulin levels in adult rats (controls and experimental groups).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Serum glucose levels (mg/dl)</th>
<th>Serum insulin levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>3 days</td>
</tr>
<tr>
<td>Normal Control (NC)</td>
<td>200±11.2</td>
<td>217.3±11.2</td>
<td>69.12±2.3</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>193±8.2</td>
<td>173±4.5</td>
<td>265±21.8***a</td>
</tr>
<tr>
<td>Diabetic+MCE (DC+MCE)</td>
<td>182±6.1</td>
<td>201±7.5</td>
<td>253±12***a</td>
</tr>
</tbody>
</table>

Values significantly different compared to normal P*** < 0.01. Values are expressed as means ± SEM. Means not sharing common letter are significantly different (p < 0.05).

TABLE 2: Triglycerides (TG) and total cholesterol (TC) levels in the serum adult rats (controls and experimental groups).

<table>
<thead>
<tr>
<th>Group</th>
<th>T.G</th>
<th>T.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (NC)</td>
<td>74.2±6.8</td>
<td>87.2±8.4</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>178.2±10.4***a</td>
<td>163.2±7.9***a</td>
</tr>
<tr>
<td>Diabetic +MCE (DC +MCE)</td>
<td>121.0±9.5b</td>
<td>110.2±3.8b</td>
</tr>
</tbody>
</table>

Values significantly different compared to normal P*** < 0.01. Values are expressed as means ± SEM. Means not sharing common letter are significantly different (p < 0.05).

Biochemistry estimation in tissue homogenates

Pancreas were removed, freed from adhering tissues and washed with ice-cold normal saline solution (0.9%). Weight of all the organs was taken only after drying the tissue. 1 g tissue was homogenized in 10 ml of 0.2 M tris-HCl with the help of homogenizer. The homogenate was filtered and then centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant obtained was used for estimation of superoxide dismutase[13], catalase[14], glutathione peroxidase[15], reduced glutathione[16] and thiobarbituric acid reactive substances[17].

Histological examination of pancreas

Some pancreas were cleaned and fixed in 10% neutral buffered formalin solution. After dehydration in graded ethanol solutions and in toluene, they were embedded in paraffin. Sections of 3–5µm thickness were stained with hematoxylin and eosin (H.E.) for histopathological examination.

RESULTS

Effects of Momordica charantia methanolic extracts on body weight, hyperglycemia and hypoinsulinemia induced by alloxan

TABLE 1 depicts the initial and final body weight, the levels of fasting blood glucose and plasma insulin in control and experimental groups of rats. Diabetic rats (alloxan) presented at the end of treatment a significant loss of body weight as compared to control ones which gained a significant weight. In addition, the levels of blood glucose were significantly increased by and those of serum insulin were significantly decreased in the diabetic rats when compared to the control group.

Oral administration of MCE to the diabetic rats for
The effects of *Momordica charantia* methanolic extract on lipid parameters are presented in TABLE 2. Our results showed that the administration of alloxan increased significantly total cholesterol (TC) and triglycerides (TG) levels after 30 days of treatment, in comparison to control rats. The administration of *Momordica charantia* methanolic extract countered the significant rise in the levels of the parameters.

**Effects of *Momordica charantia* methanolic extract on alloxan-induced lipid peroxidation and GSH content**

TABLE 3 represents the levels of MDA and GSH in pancreatic tissue of the control and experimental rats. The diabetic rats showed a significant increase in MDA levels and a decrease in GSH levels when compared to those of control group.

Increased levels of MDA were significantly declined in the diabetic rats treated with MCE associated with an increase of GSH content when compared to diabetic rats (alloxan).

**Effects of *Momordica charantia* methanolic extract on alloxan-induced changes in the antioxidant enzyme activities**

TABLE 4 The activities of enzymatic antioxidants such as SOD, CAT and GPx in the control and experimental groups of rats. The activities of these enzymatic antioxidants were significantly decreased in the diabetic rats when compared to those of control group. Oral administration of MCE to the diabetic rats showed a significant increase in the activities of SOD, CAT and GPx.

**Effects of *Momordica charantia* methanolic extract on alloxan-induced histological changes in pancreas**

**TABLE 4**: Antioxidant enzymes activities (SOD, CAT and GPx) in the pancreas tissue of adult rats (controls and experimental groups).

<table>
<thead>
<tr>
<th>Group</th>
<th>Pancreatic CAT (μ moles H2O2 decomposed/min/mg protein)</th>
<th>Pancreatic GSH-Px (μg GSH consumed/min/mg protein)</th>
<th>Pancreatic SOD (Units/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control (C)</td>
<td>265.12±15.59</td>
<td>714.26±51.23</td>
<td>321.02±31.20</td>
</tr>
<tr>
<td>Diabetic Control (DC)</td>
<td>165.24±8.49***a</td>
<td>478.23±34.36***a</td>
<td>293.58±24.19***a</td>
</tr>
<tr>
<td>Diabetic +MCE (DC+MCE)</td>
<td>321.56±17.52***a</td>
<td>645.29±65.14***a</td>
<td>338.97±29.76***a</td>
</tr>
</tbody>
</table>

Values significantly different compared to normal P*** < 0.01. Values are expressed as means ± SEM. Means not sharing common letter are significantly different (p < 0.05).
The results of *Momordica charantia* in histopathologic examination are shown in Figure 2. As revealed in (Figure 2B), a clear decrease in the area occupied by the \( \beta \) cells was observed in the pancreatic sections of alloxan-induced diabetic rats. Treatment with MCE showed a slight hypertrophy of Langerhans islets and hyperplasia in pancreas compared to alloxan treated rats (Figure 2C) revealing the protective effect of MCE.

**DISCUSSION**

Glucose is the key physiological regulator of insulin secretion; indeed, short-term exposure of \( \beta \)-cells to increasing glucose concentrations induces proliferation in a concentration-dependent manner. In addition to its effect on \( \beta \)-cell turnover, hyperglycemia also impairs \( \beta \)-cell secretory function. This glucotoxic effect is evident before apoptosis leads to a significant decrease in \( \beta \)-cell mass[18].

Alloxan-induced diabetic rats exhibited severe glucose intolerance and metabolic stress as well as hyperglycemia due to a progressive oxidative insult interrelated with a decrease in endogenous insulin secretion and release[19]. Treatment with antidiabetic drugs based on their pancreatic antioxidant activity might be a protective strategy for protecting \( \beta \)-cell due to disproportionate generation of free radicals[20,21].

The present investigation indicated that a single dose of alloxan (150 mg/kg) intraperitoneally to adult male albino rats (190-210g) was suitable to induce histological changes of the islets of Langerhans characterized appearance, hypoinsulinemia and hyperglycemic state. The present dose as well as the observed pathological and biochemical manifestations[22].

A gradual loss of \( \beta \)-cells due to apoptosis significantly hinders insulin production and inhibits cell viability. During apoptosis, cells shrink; chromatin condenses; DNA is cleaved into pieces at inter nucleosomal regions. A proactive way to increase \( \beta \)-cell viability is to decrease apoptosis level in order to retain the cell population and increase insulin production[23].

Exposure of islets to alloxan shows significantly increased formation of peroxynitrite, NO and ROS with markedly elevated lipid peroxidation and reduced cell viability. Islets exposed to alloxan also show significantly increased mitochondrial membrane potential. Apparently, alloxan causes severe oxidative and cytotoxic stress to islets that is likely to compromise their insulin releasing capacity[24].

Overproduction of ROS or exhaustion of antioxidants may cause oxidative stress which is a major factor of defective insulin secretion and increases apoptosis of pancreas[25]. Moreover, ROS produced by \( \beta \)-cell in response to metabolic stress affect mitochondrial structure and function and lead to \( \beta \)-cell failure. Specifically, ROS oxidize mitochondrial membrane phospholipids such as cardiolipin, which impairs membrane integrity and leads to cytochrome c release and apoptosis. In addition, ROS activate UCP2 via peroxidation of the mitochondrial membrane phospholipids, which results in proton leak leading to reduced ATP synthesis and content in \( \beta \)-cells[26].

However, continuous treatment of diabetic animals with MCE for 30 days caused significant decrease in levels of serum glucose, Pancreatic MDA and significant increase in serum insulin[27] as the active components of MCE, Momorcharin and Momorcidin are believed to exert their hypoglycaemic effects via different physiological and biochemical processes. These include insulin secretagogue like effect, stimulation of skeletal muscle and peripheral cell glucose utilization, inhibition of intestinal glucose uptake, inhibition of hexokinase activity, suppression of key gluconeogenic enzymes, stimulation of key enzymes, HMP pathway and preservation of pancreatic islet cells and their functions.

Similarly, alloxan caused body weight loss was also regained to its above-initial values by MCE treatment, which reflects an improved health of MCE treated animals.
The histological observation of the pancreatic tissues further substantiates the claim that MCE has a protective nature on pancreatic tissue.

The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL-cholesterol concentration and increased concentration of small dense LDL-cholesterol particles. Faulty glucose utilization causes hyperglycemia and mobilization of fatty acids from adipose tissue for energy purpose. The lipid changes associated with diabetes mellitus are attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/resistance. This results in excess fatty acid accumulation in the liver, which is converted to triglycerides. The impaired ability of insulin to inhibit free fatty-acid release leads to elevated hepatic VLDL-cholesterol production. The increased VLDL-cholesterol and triglyceride levels decrease the level of HDL-cholesterol and increase the concentration of small dense LDL-cholesterol particles by activation of lipoprotein lipase and lecithin acyl-cholesterol transferase

In our study, elevated levels of serum TC and TG concentration in alloxan-induced diabetic rats[28a]. On another hand, Induction of diabetes by alloxan resulted in loss of body weight in the diabetic control rats in[29] that may due to catabolic effect on protein metabolism by retarding protein synthesis and stimulating protein degradation. However, treatment with MCE normalized all the lipid profile parameters as MCE improves the lipid profile by modulating peroxisome proliferator-activated receptor-γ (PPAR-γ) gene expression[30]. On other hand, MCE may the lipid profile by enhancement of sympathetic activity and lipolysis[31,32].

Earlier it has been explored that oxidative stress forms the foundation for the induction of multiple cellular pathways that can ultimately lead to both the onset and subsequent complications of DM. Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation[33].

GSH oxidation is a major contributor to cell apoptosis mediated by oxidants. Accumulated evidence from our laboratory has consistently shown that an early spike in GSSG formation, typically within minutes of oxidant exposure, preceded oxidant-induced activation of mitochondrial apoptotic signaling and cell apoptosis hours later[34].

In our study, the level of pancreatic GSH was reduced in diabetic rats, which is consistent with an earlier report[35].

The decrease in tissue’s GSH content could be the result of decreased synthesis or increased degradation of GSH by oxidative stress that prevails during diabetes. Furthermore, lipid peroxidation is one of the characteristic features of chronic diabetes[28b]. In the present study, along with decreased level of reduced GSH, a marked increase in the concentration of TBARS was also observed in pancreas of diabetic mice; however, treatment of these animals with MCE decreased the elevated level of TBARS. Simultaneously, reduced GSH content was also increased significantly which indicates that the CDE can either increase the biosynthesis of GSH and/or reduce the oxidative stress that ultimately reduces the degradation of GSH. Similar results were also witnessed in an earlier study[36].

Catalase is a common enzyme found in nearly all living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen. One of the most commonly used ROS for neuronal oxidative stress preconditioning is H2O2. It is formed by the dismutation of superoxide (O2·−) spontaneously or enzymatically in a reaction that is catalyzed by superoxide dismutase (SOD). H2O2 is less reactive compared to other ROS, easily crosses membranes, and diffuses from its original site of production.

Many more studies documented a pivotal role for H2O2 in the development of vascular dysfunction in pathological conditions, such as atherosclerosis, hypertension, and diabetes mellitus. It has also been speculated that, in the vessel wall, H2O2-mediated mechanisms may compensate for the loss of NO-mediated dilation during the development of various diseases[37].

To prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. Superoxide dismutases are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen.
perroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen.

However, Oral administration of MCE causes significant increase in level of pancreatic GSH content and significant enhancement in level of and the activity of pancreatic CAT, pancreatic GSH-Px and pancreatic SOD\(^{[38]}\).

MCE may also act through inhibition of glucose absorption, enhancement of glucose disposal and recovery of impaired peripheral nerves. Firstly, \(\text{Na}^+\)- and \(\text{K}^+\)-dependent glucose absorption was reduced in the brush border membrane vesicles of the jejunum by administration of MCE. Also, MCE inhibit digestion of carbohydrate by reducing maltase activity in intestine. Secondly, MCE was able to stimulate the glucose uptake in L6 myotubes, which may be via the insulin pathway since it can be completely blocked by wortmannin, an inhibitor of PI3 kinase. Finally, the structural abnormalities of peripheral nerves were normalized with MCE in alloxan diabetic rats\(^{[39]}\). The increased activity of CAT, SOD and GSH-Px suggest a compensatory response to oxidative stress as it reduces the endogenous H2O2 produced thus diminishing the toxic effects due to this radical or other free radicals derived from secondary reactions.

Results of this study do not allow definite conclusion to be drawn on the mechanism of action of MCE in the experimental animal paradigms used. However, a number of investigators have shown that a host of various plant secondary metabolites possess hypoglycemic, hypotensive, anti-inflammatory and other pharmacological properties\(^{[40]}\). As mentioned earlier, MCE is also known to contain various secondary metabolites. Therefore, it is not unreasonable to speculate that some of these chemical compounds are presumably responsible for imparting the antihyperglycemic, antihyperlipidemic and antioxidative properties to MCE.

**CONCLUSION**

From the results obtained, it can be concluded that MCE possess significant antihyperglycemic, antihyperlipidemic and pancreatic antioxidative properties. Hence, apart from controlling hyperglycemia it would also be beneficial in the alleviation of associated diabetic complications including the prevention of the development of atherosclerosis and other coronary artery diseases. However, further studies are needed to investigate and elucidate the possible mechanism of action of the active ingredients, establish complete safety profiles and evaluate the potential value of MCE for the management of diabetes and hyperlipidemia in the clinic.

Moreover, additional parameters such as assay of fructosamine, HbA1c, C-peptide etc should also be studied. This may prove helpful for developing new drugs from this plant for managing diabetes and associated complications.

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