**ORIGINAL ARTICLE**

**In vitro alpha-amylase inhibition and in vivo antioxidant potential of *Momordica dioica* seeds in streptozotocin-induced oxidative stress in diabetic rats**

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Antioxidants

**Abstract**  
*Momordica dioica* Roxb. Commonly known as “Kakora” in Telugu, is used in the Indian traditional system of medicine for the treatment of diabetes. The aim of this study was to investigate the antidiabetic activity of methanolic extract of *M. dioica* seeds (MEMD) in streptozocin (STZ) induced diabetic rats. The *in vitro* α-amylase inhibitory activity of the MEMD was done by spectrophotometric method. Diabetes was induced by STZ (45 mg/kg; i.p), MEMD (100 & 200 mg/kg; b.wt) and standard drug metformin (50 mg/kg; b.wt) were administered to the diabetic rats. Blood glucose was estimated on the 11th day and the level of MDA, SOD and CAT was estimated in the liver tissue homogenate after the 15 days of experimental period. MEMD showed significant inhibition of alpha amylase activity and the IC₅₀ was found to be 48 μg/ml. Oral administration of MEMD significantly reduced blood glucose level (*P* < 0.05), diminished the MDA level and refurbished depleted antioxidant enzymes and Insulin level to normalcy. These findings revealed that *M. dioica* seeds possess antihyperglycemic, antioxidant and anti lipid peroxidative activity and thus mitigate STZ-induced oxidative damage.

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1. Introduction

Globally, there is a rampant increase in people affected with diabetes and pre-diabetes due to an array of clinical factors like aging, smoking, unhealthy diet, hyperlipidemia and sedentary life style (Wild et al., 2004). Based on the report cited by International Diabetes Federation, diabetes mellitus is the mammoth cause of mortality and morbidity, with a projected
range of 382 million adults being affected and 5.1 million people killed in 2013. In 2035, the incidence of the disease is ordained to be about 592 million, and the prevalence will be higher in developing countries (Africa, Asia, and South America) with low and middle-incomes. Epidemiology studies reveal that 80% of the global diabetes population is visualized in low and middle income countries (International Diabetes Federation, 2013). Diabetes is portrayed by minified glucose homeostasis which may prelude to an elevated blood glucose level with an alteration in lipid parameters (International Diabetes Federation, 2013). Meanwhile, extended accumulation of elevated blood glucose may lead to the overproduction of reactive oxygen species accompanied with oxidative stress. This noxious state may be involved in the progression of fatal pathological event like atherosclerosis, cardiovascular disease and other diabetic complications. Further, uncontrolled elevated glucose level may impose a greater burden in the form of restricted daily activity and work which result in high economic costs (Juárez-Reyes et al., 2015).

Albeit, the allopathic medicines are currently used in the management of diabetes mellitus, they exert serious adverse effects and affect the quality of life (Aicher et al., 2010). Nowadays, herbal remedies are gaining more attention in the treatment of a wide range of diseases as they have minimal adverse effects. Thus, herbal medicines encompassing anti hyperglycemic potential may serve as a suitable and safer alternative or as an adjunct candidate in the management of hyperglycaemia. Ethnopharmacological reports authenticate that a wide array of plants are used in folkloric medicine for their purported anti-diabetic activity (Gupta et al., 2005a,b; Kesari et al., 2005). The anti-diabetic efficacy in a wide range of plants and plant products has been proved in various preclinical models (Gupta et al., 2005a,b; Kesari et al., 2006; Mapanga and Musabayane, 2010) as well as in human beings (Jayawardena et al., 2005; Musabayane, 2012). In India, several indigenous plant products have been used by practitioners in the Ayurvedic system to treat hyperglycemia. *M. dioica* Roxb. EX. Wild is a wild plant of the genus Momordica. In folklore culture, *M. dioica* fruits, leaves, and tuberous roots are used for the treatment of diabetes mellitus in India (Sadyojatha and Vaidya, 1996). Previous preclinical reports prove the anti diabetic activity and nephroprotective properties of *M. dioica* (Gupta et al., 2011; Reddy et al., 2006). However, there is a lack of credible evidence to reveal the antidiabetic activity of *M. dioica* seeds. In this notion, the present study entails the invitro anti amylase and antidiabetic activity in streptozocin (STZ) induced hyperglycemic rats.

2. Materials and methods

2.1. Plant extraction

Fresh fruits of *M. dioica* were collected locally, during July–August, 2011 and seeds were removed mechanically and dried under shade. They were identified and authenticated by Sri Venkateshwara University, Botany department, Tirupati. The seeds were powdered in an electric grinder. The coarse powder was subjected to methanol extraction, in Soxhlet apparatus and was run about 10 cycles. Then the resultant product is filtered, dried in a desiccator and stored until further use.

2.2. Chemicals and reagents

Streptozotocin was purchased from Sisco Research laboratories Pvt Ltd. Acarbose was purchased from Glucobay, Bayer Pharma, India. Metformin was a gift sample from Ranbaxy Pvt Ltd, Punjab, India. Other chemicals and reagents used in the study were of high purity.

2.3. Preliminary phytochemical screening

MEMD was screened for the presence of various phytoconstituents like alkaloids, flavonoids, steroids, tannins, glycosides, triterpenoids and saponins.

2.4. Invitro α-amylase inhibitory assay

The α-amylase inhibitory activity of MEMD was estimated by the method of Bernfeld using Acarbose as the standard (Bernfeld, 1955). Briefly a series of concentrations of MEMD (10, 40 and 80 μg/ml) was prepared and allowed to react with α-amylase and 2 mM of phosphate buffer (pH- 6.9). After incubation for 20 min, to the reaction mixture 0.1 ml of 1% starch solution was added. The same procedure was followed for control samples without the enzyme. Finally, 0.5 ml of dinitro salicylic acid reagent was added to both control and test and kept in a boiling water bath for 5 min. Then, the absorbance was measured at 540 nm using spectrophotometer and the percentage inhibition was calculated using the formula,

$$\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}/\text{Absorbance}_{\text{control}} \times 100$$

2.5. Animals

Male Wistar rats weighing about 150–200 g were obtained from National Institute of Nutrition, Hyderabad. Animal Protocol was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for Purpose of Control and Supervision of Experimentation on Animals) through its reference no: IAEC/SVCP/2011/006, Dated: 26/7/11. Animals were kept for acclimatization at an ambient temperature of 25°C and 45–55% relative humidity, with 12 h each of dark and light cycles and were fed pelleted diet and water ad libitum.

2.6. Acute toxicity studies

The acute toxicity studies for MEMD were done according to the OECD guidelines No. 423. The extract did not produce any signs of toxicity when given in doses up to 2000 mg/kg by an oral route. Hence, for further studies 100 & 200 mg/kg dose of the extract were selected.

2.7. Streptozocin induced diabetes

A freshly prepared solution of STZ (45 mg/kg in 0.1 M citrate buffer, pH 4.5) was injected intraperitonially to overnight-fasted rats The rats exhibited hyperglycemia within 48 h of STZ administration (Vogel et al., 2002). The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.
2.8. Experimental design

The experiment was carried out in five groups of six rats each:

Group I- Normal control rats received saline.
Group II- Diabetic control.
Group III- Diabetic rats treated with standard drug, Metformin (50 mg/kg).
Group IV- Diabetic rats treated with MEMD (100 mg/kg in 0.5% CMC).
Group V- Diabetic rats treated with MEMD (200 mg/kg 0.5% CMC).

At the end of the experimental period, the blood was withdrawn by retro orbital puncture and centrifuged. Serum glucose was estimated on the 11th day at 0 h, 1 h, 2 h and 4 h by Glucose oxidase method and the absorbance was measured at 505 nm by UV-Spectrophotometer (ELICO-Sl-159). The fasting serum insulin was estimated by Maglumi auto analyzer (MAGLUMI 1000, Fully auto chemiluminescence Immunoassay (CLIA) analyzer at Qedcure labs, Kukatpally, Hyderabad (Hagino et al., 1994). On the 16th day animals were sacrificed and the liver was removed and homogenized for in vivo antioxidant activity study.

2.9. Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris–HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 min, supernatant was collected and used for various in vivo antioxidant assays.

2.10. Invivo antioxidant activities

Lipid Peroxidation is based on the reaction of Malondialdehyde (MDA) one of the products of lipid peroxidation with thiobarbituric acid to form Thiobarbituric acid reactive substances (TBARS), which have a pink color with absorption maxima at 540 nm (Okhawa et al., 1979). Catalase (CAT) activity was determined according to the method of Aebi (1983). Superoxide dismutase (SOD) activity was determined colorimetrically by the method of Kono (1978).

3. Statistical analysis

Results were expressed as mean ± S.E.M. For statistical analysis of the data group, mean was compared by a one-way analysis of variance (ANOVA) followed by Dunnett’s test. p < 0.001 was considered to be statistically significant.

4. Results

4.1. Acute toxicity studies

Acute oral toxicity studies revealed the non toxic nature of MEMD. There was no lethality observed nor any profound toxic reactions found at a dose of 2000 mg/kg b.wt. p.o. which indirectly pronounces the safety profile of the plant extract.

4.2. Preliminary phytochemical analysis

Preliminary phytochemical analysis showed the presence of alkaloids, flavanoids, steroids, tannins, glycosides, triterpenoids and saponins in MEMD.

4.3. Effect of MEMD on α-amylase activity

In the present study, MEMD displayed a significant inhibition α-amylase enzyme in a concentration dependent manner. MEMD at the concentrations 10, 40 & 80 µg/ml elicited 11.33%, 42.6% and 75% of α-amylase enzyme inhibition respectively. The IC50 value was found to be 48 µg/ml. Meanwhile, the standard compound Acarbose showed 6%, 27% and 80% of inhibition at the same concentration used in the test and IC50 value was 35 µg/ml (Fig. 1).

4.4. Effect of MEMD on blood glucose and insulin levels

STZ intoxicated rats displayed a significant (p < 0.001) elevation of blood glucose level in 0, 1, 2 & 4 h as compared to the control rats. However oral administration of MEMD (100 mg/kg) failed to diminish the blood glucose level in 1, 2 & 4 h as compared to the STZ rats (p > 0.05). While, in the case of MEMD (200 mg/kg) a significant (p < 0.001) reduction in blood glucose level was observed in 1, 2 & 4 h as that of diabetic induced rats (Table 2). Fasting insulin levels in MEMD treated diabetic rats restored the levels significantly (p < 0.0001) to normalcy as compared to diabetic control rats (Table 1).

4.5. Effect of MEMD and STZ on lipid peroxidation

In the present study, STZ induced diabetic rats displayed a significant (p < 0.001) elevation of MDA, a reliable marker lipid peroxidation in the hepatic tissue homogenate, while, treatment with MEMD (100 & 200 mg/kg) significantly (p < 0.001) reduced the MDA level to normalcy (Fig. 2).

4.6. Effect of MEMD and STZ on antioxidants level in hepatic tissue

The SOD and CAT levels were significantly (p < 0.001) reduced in the liver of STZ induced diabetic rats. Oral intubation of MEMD (200 mg/kg) significantly restored the

Figure 1 Effect of methanolic extract of M. dioica (MEMD) on in vitro α-amylase activity. The data are expressed as mean ± S.E. M. n = 3 in each group.
Depleted (p < 0.001) antioxidant level to normalcy. MEMD (100 mg/kg) doesn’t per se any significant effects on the antioxidant level (Fig. 3).

5. Discussion

Diabetes mellitus is an alarming metabolic disorder leading to hyperglycemia which later develops to micro- and macrovascular complications and becomes a major cause of death. α-amylase is a digestive enzyme attached to the membrane of brush border of the small intestine. It orchestrates a catalytic conversion of complex carbohydrates into monosaccharides that can be absorbed. Reduction of postprandial hyperglycemia is the mainstay in the treatment paradigm of diabetes mellitus. Blood glucose level reduction can be confronted by extending the glucose absorption through the downregulation of carbohydrate hydrolyzing enzymes in the digestive tract. α-amylase inhibitors play a pivotal role in blocking the release of glucose from dietary source of carbohydrates and extend glucose absorption leading to decreased postprandial plasma glucose levels and further minify postprandial hyperglycemia (Lebovitz, 1997). Multitude plant extracts and natural products decrease the glucose production from carbohydrate in the gut or glucose absorption from the intestine. In our study, MEMD effectively inhibited the α-amylase activity in vitro which is in line with a previous report (Adisakwattana and Chanathong, 2011).

Streptozocin (STZ) is a noxious chemical agent used to induce a state of hyperglycemia in preclinical models. The mechanism of STZ induced diabetes is mainly due to the rampant generation of reactive oxygen species (ROS), since STZ intoxication causes rapid ATP dephosphorylation which may lead to substrate availability for xanthine oxidase. So, these series of toxic events lead to the generation of superoxide radicals, hydrogen peroxide, and hydroxyl radicals and cause oxidative pancreatic β-cell damage (Szkudelski, 2001). Further, STZ causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO). This results in a rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β-cell necrosis (Haluzík and Nedvídková, 2000). In the present study, STZ significantly induced hyperglycemia and oral administration of MEMD for a period of 10 days caused a significant decrease in blood glucose levels. The promising mechanism by which MEMD mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals.

Table 1 Effect of methanolic extract of M. dioica (MEMD) on blood glucose level in diabetic rats.

<table>
<thead>
<tr>
<th>Treatment and dose</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.4 ± 3.80</td>
<td>99.83 ± 3.01</td>
<td>108.5 ± 1.48</td>
<td>82.97 ± 0.85</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>268.75 ± 5.5</td>
<td>272.22 ± 3.95</td>
<td>280.95 ± 3.26</td>
<td>281 ± 4.78</td>
</tr>
<tr>
<td>DC + MEMD (100)</td>
<td>290 ± 5.59</td>
<td>280 ± 4.72</td>
<td>272.6 ± 3.90</td>
<td>271.5 ± 3.80</td>
</tr>
<tr>
<td>DC + MEMD (200)</td>
<td>300.83 ± 12.34</td>
<td>204.66 ± 2.4</td>
<td>173.33 ± 6.14</td>
<td>161.66 ± 5.57</td>
</tr>
<tr>
<td>DC + Metformin (50)</td>
<td>280.07 ± 15.36</td>
<td>262.5 ± 8.54</td>
<td>153.34 ± 2.48</td>
<td>138.33 ± 1.82</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± S.E.M.; n = 6 in each group. *p < 0.0001, highly significant, compared to diabetic control.

Table 2 Effect of methanolic extract of M. dioica (MEMD) on serum insulin level in diabetic rats.

<table>
<thead>
<tr>
<th>Treatment and dose</th>
<th>Insulin levels (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.74 ± 0.41</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3.50 ± 0.73</td>
</tr>
<tr>
<td>DC + MEMD (100)</td>
<td>3.01 ± 0.36</td>
</tr>
<tr>
<td>DC + MEMD (200)</td>
<td>7.58 ± 0.72</td>
</tr>
<tr>
<td>DC + Metformin (50)</td>
<td>10.67 ± 0.73</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± S.E.M.; n = 6 in each group. *p < 0.0001, highly significant, compared to diabetic control.

Figure 2 Effect of methanolic extract of M. dioica (MEMD) on lipid peroxidation. The data are expressed in mean ± S.E.M. n = 6 in each group p < 0.0001, highly significant, compared to diabetic control.

Figure 3 Effect of methanolic extract of M. dioica (MEMD) on antioxidant enzymes. The data are expressed as mean ± S.E.M. n = 6 in each group. *p < 0.0001, highly significant, compared to diabetic control. Units: CAT: U/mg of protein; SOD: μmoles/mg of protein.
In diabetes, tissue damage is considered to be mediated by free radicals by attacking membranes through peroxidation of unsaturated fatty acids (Ravi et al., 2004). Lipid peroxidation eventually leads to extensive membrane damage and dysfunction (Alfy et al., 2005). The decreased lipid peroxidation and improved antioxidant status may be one of the mechanisms by which drug treatment could contribute to the prevention of diabetic complications (Kamalakkannan and Prince, 2006). In our study, MEMD significantly attenuated the increased lipid peroxidation which could be due to the antioxidant effect of flavonoids, detected in the preliminary phytochemical screening of the extract.

The lipid peroxidation induced by diabetes displays a substantial decrease in the level of prime antioxidant enzymes like SOD and CAT. These biomolecules have significant functions in quenching the free radicals and dysfunctioning the toxic adduct formation during the lipid peroxidation process. On the other hand, the depleted level of antioxidant enzymes may prelude to an elevated level of noxious superoxide anion (O$_2^-$) and hydrogen peroxide in the vital tissues. These radicals overture to release hydroxyl radicals, resulting in the initiation and propagation of lipid peroxidation (Latha and Pari, 2003). The MEMD treatment increased the activity of enzymes and may thereby help to control free radicals. In this context, the antidiabetic potential MEMD might be due to the effect of bioactive principles present in the plant. Previous literature highlights the isolation of isoflavone, daidzein in the fruit of M. dioica (Kale and Laddha, 2012). Further, Momodicaursenol, an unknown pentacyclic triterpene was also reported in the seeds of M. dioica (Ali and Srivastava 1996). Thus, the antidiabetic activity of MEMD in the present study might be due to the presence of these phytoconstituents.

In conclusion, the hypoglycemic action of M. dioica seeds has beneficial effects on blood glucose level which might be mediated through its α-amylase inhibitory activity. Meanwhile, lipid peroxidation was significantly reduced and the level of endogenous antioxidant enzymes (CAT and SOD) was boosted. Thus, the anti diabetic of M. dioica seeds might be due to free radical scavenging and antioxidant activity. Further, molecular studies and isolation of the active component in the extract was highly warranted to elucidate the mechanism of action of the plant.

References


