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# Extract of *Moringa oleifera* leaves ameliorates streptozotocin-induced *Diabetes mellitus* in adult rats

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ABSTRACT

Medicinal plants attract growing interest in the therapeutic management of *Diabetes mellitus*. *Moringa oleifera* is a remarkably nutritious vegetable with several antioxidant properties. The present study assessed the possible antioxidant and antidiabetic effects of an aqueous extract of *M. oleifera* leaves in treating streptozotocin-induced diabetic albino rats. The antidiabetic effects of aqueous extract of *M. oleifera* leaves were assessed histomorphometrically, ultrastructurally and biochemically. Fasting plasma glucose (FPG) was monitored and morphometric measurements of β-cells of islets of Langerhans (modified Gomori's stain) and collagen fibers (Mallory's trichrome stain) were performed. The antioxidant effects of *M. oleifera* leaves were determined by measuring the reduced glutathione and lipid peroxidation product, malondialdehyde, in pancreatic tissue. *M. oleifera* treatment significantly ameliorated the altered FPG (from 380% to 145%), reduced glutathione (from 22% to 73%) and malondialdehyde (from 385% to 186%) compared to control levels. The histopathological damage of islet cells was also markedly reversed. Morphometrically, *M. oleifera* significantly increased the areas of positive purple modified Gomori stained β-cells (from 60% to 91%) and decreased the area percentage of collagen fibers (from 199% to 120%) compared to control values. Experimental findings clearly indicate the potential benefits of using the aqueous extract of *M. oleifera* leaves as a potent antidiabetic treatment.



## Introduction

*Diabetes mellitus* (DM) is a prevalent chronic disease in many countries. Changing lifestyles, reduced physical activity and increased obesity contribute to the increasing the number of patients with DM (Shaw et al., 2010). The total number of diabetic patients worldwide has been estimated to increase from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004), though this figure was already reached by 2011 according to the International Diabetes Federation (IDF). Moreover, projections of DM incidence in 2030 could well reach 530 million people on a global scale and 8.6 million people in Egypt (Arafa and Amin, 2010).

Although the etiology of this disease is not well defined, viral infection, autoimmune disorder, and environmental factors have been implicated (Shewade et al., 2001). Increased oxidative stress, impaired antioxidant defense systems and consequently lipid peroxidation are major participants in the development and progression of DM and its complications (Rudge et al., 2007). The therapeutic management of DM with minimal side effects remains a clinical challenge. There is growing interest in the potential use of medicinal plants as an alternative treatment for diabetes as these are commonly cheaper, less toxic and with fewer side effects (Nissen and Wolski, 2007).

Streptozotocin (STZ) is a broad spectrum antibiotic and alkylating genotoxic agent which possesses antibacterial, tumoricidal, carcinogenic and diabetogenic properties (LeDoux et al., 1986; Van Dyke et al., 2010). Induction of experimental diabetes in rats using streptozotocin is very simple to do and provides a convenient model to study the activity of hypoglycemic agents (Ar'Rajab and Ahren, 1993; Brenna et al., 2003). Moreover, it can be used to study various consequences of DM such as osteopenia (Musumeci et al., 2011). It is specifically toxic to pancreatic β-cells involving uptake by glucose transporter 2 (GLUT-2) (Vessal et al., 2003). STZ impairs glucose oxidation and decreases insulin biosynthesis and

*Abbreviations:* DM, *Diabetes mellitus*; FPG, fasting plasma glucose; GSH, reduced glutathione; LPO, lipid peroxidation; MDA, malondialdehyde; *M. oleifera*, *Moringa oleifera*; PPPG, post-prandial plasma glucose levels; ROS, reactive oxygen species; STZ, streptozotocin.

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secretion (Nukatsuka et al., 1990). It also generates reactive oxygen species (ROS), which contribute to DNA fragmentation and evokes other deleterious changes in β-cells (Takasu et al., 1991; Fukudome et al., 2008). When injected into adult rats, streptozotocin can cause Type-1 DM with severely elevated blood glucose levels, however, when administered to neonatal rats, the neonates develop Type-2 DM (Takada et al., 2007).

*Moringa oleifera* Lam (Moringaceae, *M. oleifera*) is a highly nutrient-rich plant with exceptional medicinal properties widely used to treat various health care problems (Farooq et al., 2012). It provides a rich combination of nutrients, amino acids, antioxidants, anti-aging and anti-inflammatory compounds and is employed as medication for a variety of ailments particularly in South Asia and India. Since 1998, the World Health Organization has promoted *Moringa* as an alternative to imported food supplies to treat malnutrition. In addition to the important medicinal properties and high nutritional value of various parts of this plant such as leaves, roots, seeds, bark, fruit, flowers and immature pods, it also has a potent water purifying property (Anwar et al., 2007). Not surprisingly, *M. oleifera* is sometimes described as "Mother's Best Friend", "Miracle Tree", "Tree of Life" and "God's Gift to Man" (Johnson, 2005; Mbikay, 2012).

*M. oleifera* is cultivated in many tropical and subtropical countries of Asia and Africa. It is commonly known there as the drumstick tree or horseradish tree (Mbikay, 2012), while in the Nile valley, its name is 'Shagara al Rauwaq', which means 'tree for purifying' (Von Maydell, 1986). It is used as a leafy vegetable with leaves that can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and without loss of nutritional value (Anwar et al., 2007). The therapeutic effects of *M. oleifera* leaves have been attributed to the combined actions of various bioactive components found in the plant that include trace metal ions, especially potassium, calcium, phosphorous, zinc, manganese and iron, vitamins A, D, E and C (Aslam et al., 2005; Gowrishankar et al., 2010), alkaloids such as moringinine, carotenoids such as β-carotene and essential amino acids (Amaglo et al., 2010). Moreover, *M. oleifera* contains three structural classes of phytochemicals which have several medicinal benefits. They are glucosinolates such as glucomoringin, flavonoids such as quercetin and kaempferol and phenolic acids such as chlorogenic acid (Mbikay, 2012). These phytochemicals have been reported to possess antioxidant, hypoglycemic, hypotensive, antidyslipidemic, anticancer, and anti-inflammatory properties (Bennett et al., 2003; Lako et al., 2007; Manguro and Lemmen, 2007; Amaglo et al., 2010; Kasolo et al., 2010).

*High-performance liquid chromatography* (HPLC) and mass spectrometry (MS/MS) analysis of the aqueous extract of leaves of *M. oleifera* demonstrated that the leaves have a total phenolic content of 105.04 mg gallic acid equivalents (GAE)/g), total flavonoids content of 31.28 mg quercetin equivalents (QE)/g), and ascorbic acid content (106.95 mg/100 g). Moreover, *M. oleifera* leaves have demonstrated better antioxidant, anti-free radicals and inhibition activities of lipid peroxidation and protein oxidation when compared with standard α-tocopherol (Singh et al., 2009).

Most of the reviewed literature reported changes in the biochemical parameters of DM after short term treatment with extract of *M. oleifera* leaves (Ndong et al., 2007; Jaiswal et al., 2009, 2013) though only relatively few studies have described the related histological pancreatic changes (Gupta et al., 2012). On the other hand, no reviewed data have described the detailed histological changes after long-term ingestion of extract of *M. oleifera* leaves. It has been reported that *M. oleifera* medication given to diabetic patients can induce better glucose tolerance by extending the treatment period (Ghiridhari et al., 2011). Therefore, the current research study was directed toward the identification of the chronic antioxidant and antidiabetic properties of an aqueous extract of *M. oleifera* leaves against experimental streptozotocin-induced *Diabetes mellitus* in rats using histomorphometrical, histochemical and ultrastructural techniques.

## Materials and methods

### Plant collection

Fresh young leaves of *M. oleifera* (about two years old) were collected, two weeks before the beginning of the experiment, from the "Agricultural Development Corporation farms" in the Valley of Senor, Beni Suef Province, Egypt. Botanical identification and authentication was conducted by Prof. Dr. Ahmad Akl, Horticulture Department, Faculty of Agriculture, Minia University, Egypt.

### Extract preparation

Fresh leaves of *M. oleifera* were washed under running water, air dried and then ground to powder and kept dry in an air-tight container prior to the extraction. The plant derived aqueous extract tested in this study was prepared in the laboratory by mixing 1 g dried and powdered leaves with 10 ml boiling water for 15 min. The mixture was then filtered twice through a 2 μm pore sterile filter paper into a sterile tube and left to cool. The aqueous extract stock solution (100 mg/ml) was freshly prepared each day before animal ingestion (Berkovich et al., 2013).

### Animals and experimental protocols

Forty adult normoglycemic male albino rats (Sprague-Dawley) weighing 180–200 g, twelve months old, were used in the present work. Rats were provided by Kasr-Al-Aini, Faculty of Medicine, Cairo University Animal House and bred in specific pathogen-free condition. They were maintained in an air-conditioned room (20–25 °C) and subjected to a 12:12-h day light/darkness cycle with free access to food and water. All the ethical protocols and guidelines for animal handling and treatment were followed and supervised by the animal facilities, Faculty of Medicine, Cairo University in compliance with the national standards published in the Guide for the Care and Use of Laboratory Animals.

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ, Sigma–Aldrich, St. Louis, MO, USA) at a dose of 60 mg/kg BW in 0.1 mol/L citrate buffer (pH 4.5) to overnight fasted rats (Howarth et al., 2005; Akbarzadeh et al., 2007). Seventy-two hours after STZ treatment, DM development in the experimental groups was confirmed by measuring fasting plasma glucose (FPG) levels of blood samples from the tail vein of overnight fasted rats. Rats exhibiting FPG of 250 mg/dL or higher were considered to be diabetic and were included in the present study. This day was considered the first day of the experiment.

Rats were divided into four groups ($n = 10$ per group): (1) The Control group received standard diet only; (2) The Sham control group diet was supplemented daily for eight weeks with 200 mg/kg aqueous extract of *M. oleiefera* leaves; (3) STZ group; (4) *M. oleiefera*-STZ group in which the diabetic animals were treated with aqueous extract of *M. oleiefera* leaves at a dose of 200 mg/kg, the optimal dose (Jaiswal et al., 2009, 2013; Awodele et al., 2012). The extract was ingested via gastric intubation and at the end of application the gavage tube was left for several seconds to avoid regurgitation and to assure supplying the total calculated dose. The extract was given once daily at a fixed time for the whole eight-week period of the experiment. By the end of the experiment all rats were deprived of food overnight and sacrificed by $CO_2$ narcosis. Blood samples were