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Influence Of Moringa Oleifera Seeds Oil In Liver Protection And Blood Lipid Reduction In Diabetic Rats

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Abstract

The present study has been conducted to estimate the influence of Moringa oleifera seeds oil in protection the liver and reduction of blood lipids in diabetic rats. Forty male rats were divided into four groups. Non-diabetic rats fed standard diet only (G1), non-diabetic rats fed standard diet + Moringa seeds oil (1.8 mg/kg) (G2), diabetic rats fed standard diet (G3) and diabetic rats fed standard diet + Moringa seeds oil (1.8 mg/kg) (G4). The study conducted for four weeks. The results showed that liver weight and relative liver weight were significantly increased in diabetic rats (G3) compared with (G1) and significantly decreased in diabetic rats treated with Moringa seeds oil (G4) compared with (G3). Treatment diabetic rats with Moringa seeds oil (G4) showed significantly reduction in serum glucose level comparing with non-treated diabetic rats (G3).The diabetic rats (G3) showed significantly elevation in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), low-density lipoprotein (LDL), total cholesterol(CHOL) and significant decreased in high-density lipoprotein (HDL) compared with (G1). Diabetic rats treated with Moringa seeds oil (G4) showed a significant reduction in liver enzyme markers and improved in lipid profile parameters compared to non-treated diabetic rats (G3).The study concluded that Moringa seeds oil can be protect the liver and reduce blood lipids in diabetes. The potential mechanism of Moringa seeds oil in protection the liver possibly resulting from ability of free radical scavenging by antioxidants in the oil.

Keywords: Diabetes mellitus, Moringa oleifera seeds oil, liver enzymes, lipid profile.

Introduction:

Diabetes Mellitus (DM) is a worldwide general medical issue with increasing the incidence rate and prevalence, especially in developing and recently industrialized nations (**Danaei et al., 2011**). The number of people with diabetes is rising every day. Currently, diabetes is evaluated to influence around 422 million adults worldwide (**World Health Organization, 2016**). The greater concern in diabetes is the serious complications associated with it and resulting from it, which can affect many vital organs, such as nephropathy, neuropathy, retinopathy, hepatopathy and cardiovascular diseases (**Feldman et al., 2016**).

Liver diseases that occurring as a consequence of diabetes mellitus such as abnormal glycogen deposition, non-alcoholic fatty liver disease, cirrhosis, hepatocellular carcinomas, fibrosis, abnormal elevated hepatic enzymes , acute liver disease, biliary disease, cholelithiasis and cholecystitis (**Levinthal and Tavill ,1999**). Furthermore, an excessive aggregation of fat in the liver may lead to insulin resistance and acute metabolic dysfunction. A fatty liver and hyperglycaemia can damage the liver cells and lead to increased morbidity and mortality rate in diabetics (**Güven et al., 2006**). In diabetes mellitus, there is increased formation of reactive oxygen species which cause a chain of reactions leading to the peroxidation of lipids, lipoprotein alteration, and several cellular mutations of biomolecules. This leads to oxidative stress which is an important risk factor in the pathogenesis of chronic diseases such as diabetic and its complications (**Forbes and Cooper, 2013**). Oxidative stress is a major factor in diabetic pathophysiology leading to dyslipidemia, impaired glucose tolerance, beta-cell dysfunction, and resulting to liver malfunction (**Tangvarasittichai, 2015**).

Moringa oleifera (MO) is a fast-growing softwood tree indigenous to Himalaya. Nowadays, is mainly found in the Middle East, in African and Asian countries. It is spreading to other areas; due to its adaptability. All parts of the Moringa tree (leaves, flowers, roots and seeds) are suitable for human consumption (**Leone et al., 2015**). Moringa oleifera seeds contain a significant amount of oil (> 40%) with

a high - quality fatty acid composition (oleic acid > 70%), a notable resistance to oxidative degradation after refining (**Anwar et al., 2005**). Moringa oleifera seeds oil has light yellow color, with pleasant nutty flavor. Due to lower levels of peroxides and pleasant nutty flavor of Moringa oleifera seeds oil, it is normally used without any pre-processing (refining, bleaching and deodorization), which is mandatory for most of the commercial vegetable oils (**Abdulkarim et al., 2006**). Moringa oleifera seed oil is characterized by high tocopherol content. The average content of α -tocopherol, which has the greatest vitamin E potency, reaches 132.3 mg/kg. (**Manzoor et al., 2007**). Moreover, it is a rich source of vitamin A and E with strong antibacterial properties. It also possesses antihypertensive, antifungal and antiepileptic characteristics (**Pauwels, 2011**).

The seed extract of Moringa oleifera has been found to possess good antimicrobial activity against numerous bacterial and fungal species. Many of the phytochemical compounds isolated from the seeds are able to inhibit the growth of certain pathogenic microorganisms responsible for human infections (**Govardhan et al., 2013**). Moringa oleifera seeds have been found to be good antioxidants, able to reduce oxidative damage associated with aging and cancer. Many of the bioactive compounds isolated from seeds have been found to be potential antitumor promoters (**Al-Asmari et al., 2015**). Several studies have found that Moringa oleifera seed oil have good antioxidant activity and have isolated phytochemical compounds (**Singh et al., 2009**).

Moringa seeds and oil are interesting products for their nutritional composition and their content of bioactive compounds. This study is carrying out to evaluate the effect of Moringa oleifera seeds oil in protection the liver and reduction of blood lipids in diabetic rats.

Materials and Methods:

Experimental animals:

Experimental animals used for this study were forty adult male albino rats of Spague Dawley Strain weighting (150-200 g). Standard diet was prepared according to (**Philp et al., 1993**). All rats allowed free access to water and standard diet for seven days as an adaptation period before starting the experiment.

Induction of diabetes:

Diabetes was induced in rats by injecting intravenously with freshly prepared Streptozotocin from (Sigma- Aldrich), (STZ; 60 mg/kg body weight in 0.1mol/L citrate buffer, pH 4.5) (**Jaiswal et al., 2013**).

Experimental design:

Forty rats were divided into four groups (10 rats each) as follows:

Group 1: (-ve control) non-diabetic rats fed standard diet only.

Group 2: non-diabetic rats fed standard diet containing Moringa seeds oil (1.8 mg/kg body weight).

Group 3: (+ ve control) diabetic rats fed standard diet only.

Group 4: diabetic rats fed standard diet containing Moringa seeds oil (1.8 mg/kg body weight).

The study was conducted for four weeks. The rats were weighed at start of the study and weekly. Rats were weighed at the end of experimental, fasted overnight and sacrificed under anesthesia. Blood samples of rats were centrifuged at 3000 r.p.m. for 15 minutes. Serum samples were carefully separated and stored at -20 °c for biochemical analysis.

Experimental Analysis:

Determination of liver weight and relative liver weight:

After sacrificed of rats and blood sampling, liver was removed, rinsed in saline (0.9 % Na Cl) and weighed. The relative weigh of liver was estimated by comparing the organ weight to the total body weight of each rat.

Determination of glucose level:

Fasting blood sugar determined using the method of (**Bergmeyer and Bernt, 1974**), using a kit produced by Spectrum (Egyptian Company for Biotechnology).

Determination of liver enzymes:

Serum alanine aminotransferase (ALT) activity was estimated using the method of (**Srivastava et al., 2002**). Serum aspartate aminotransferase (AST) activity was estimated using the method of (**Schumann and Klauke, 2003**). Serum alkaline phosphatase (ALP) activity was estimated using the method of (**Tietz and Shuey, 1986**). All assays were analyzed using diagnostic kits produced by Spectrum (Egyptian Company for Biotechnology).

Determination of lipid profile levels:

Low-density lipoprotein (LDL) was estimated using the method of (Okada, 1996). High-density lipoprotein (HDL) was estimated using the method of (National Cholesterol Education Program, 1995). Total cholesterol (CHOL) was estimated using the method of (Ellefson and Caraway, 1976).

Statistical analysis:

The results were analyzed statistically using SPSS program. Values are represented as mean \pm SD (standard deviation). One way analysis of variance (ANOVA) was applied (Waller and Duncan, 1969). $P < 0.05$ was considered statistically significant.

Results and Discussion:

As shown in table (1) the liver weight and the relative liver weight of diabetic rats (G3) significantly increased compared with non-diabetic rats (G1). While, there were significant decrease in liver weight and the relative liver weight in diabetic rats treated with Moringa seeds oil (G4) when compared with diabetic group (G3). These results consistent with the results of (Omodanisi *et al.*, 2017) who demonstrated that oral administration of methanolic extract of Moringa to diabetic rats showed significantly reduction in liver weight and relative weight liver when compared to diabetic control group.

Non-treated diabetic rats (G3) showed significantly elevation in serum glucose level compared with non-diabetic rats (G1). Treatment diabetic rats with Moringa seeds oil (G4) showed significantly reduction in serum glucose level comparing with non-treated diabetic rats (G3).

The results consistent with (Szkudelski, 2001 and Gajdosik *et al.*, 1999) who showed that the Streptozotocin (STZ) produces pancreatic islet β -cell destruction and causes a persistent disease state characterised by severe hyperglycemia with major clinical signs of diabetes mellitus. Moreover, the results are agree to other studies, (Al Malki and El Rabey 2015) which confirmed that treated diabetes rats with Moringa seeds powder showed an excellent antidiabetic activity due to its content of antioxidant compounds and recovered the diabetic rats to the normal healthy status. Also, (Luangpiom *et al.*, 2013 and Toma *et al.*, 2012) who reported that treatment diabetic rats with Moringa oleifera reduced glucose level which indicating of amelioration in impaired glucose metabolism.

Table (1): Effect of treating diabetic rats with *Moringa oleifera* seeds oil on liver weight, relative liver weight and serum glucose level.

Parameters	G1(-ve) Nondiabetic rats	G2 Nondiabetic rats + <i>Moringa oleifera</i> seeds oil (1.8 mg/kg)	G3 (+ve) Diabetic rats	G4 Diabetic rats + <i>Moringa oleifera</i> seeds oil (1.8 mg/kg)
liver weight (g)	7.42 ± 0.23	7.48 ± 0.25 ^c	8.62 ± 0.30 _{ab}	7.16 ± 0.58 ^c
Relative liver weight %	3.37 ± 0.11	3.42 ± 0.07 ^c	5.54 ± 0.16 _{ab}	3.70 ± 0.12 ^{abc}
Glucose (mg/dl)	85.82±2.84	87.41±2.71 ^c	265.52±2.14 _{ab}	150.46±2.63 _{abc}

Values are represented as mean ± SD of 10 rats per group. ^(a) values are significant compared with nondiabetic rats. ^(b) values are significant compared with nondiabetic rats + moringa. ^(c) values are significant compared with diabetic rats.

The obtained result in table (2) indicated that the activities of serum liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were significantly elevated in diabetic rats (G3) compared with non-diabetic rats (G1). While, diabetic rats treated with *Moringa* seeds oil (G4) showed significantly reduction in the increased activities of serum liver marker enzymes in comparison with non-treated diabetic rats (G3).

Table (2): Effect of treating diabetic rats with Moringa oleifera seeds oil on liver enzymes.

Parameters	G1(-ve) Nondiabetic rats	G2 Nondiabetic rats + Moringa oleifera seeds oil (1.8 mg/kg)	G3 (+ve) Diabetic rats	G4 Diabetic rats + Moringa oleifera seeds oil (1.8 mg/kg)
ALT (U/L)	36.43±1.03	34.52±1.23 ^c	69.70±0.99 ^{ab}	49.57±0.89 ^{abc}
AST (U/L)	69.03±3.43	67.13±2.03 ^c	128.63±5.63 ^{ab}	75.73±3.74 ^{abc}
ALP (U/L)	113.53±4.04	111.03±5.14 ^c	157.20±6.73 ^{ab}	127.64±2.23 ^{abc}

Values are represented as mean ± SD of 10 rats per group. ^(a) values are significant compared with nondiabetic rats. ^(b) values are significant compared with nondiabetic rats + moringa. ^(c) values are significant compared with diabetic rats.

Diabetes is associated with liver abnormalities known as non-alcoholic fatty liver disease (NAFLD). NAFLD is a clinic histopathological diagnosis characterized by hepatocellular steatosis which is usually macro vesicular (Brunt, 2001). Liver injury is a serious complication in diabetic patients (Mohamed *et al.*, 2016). The primary and most important indicators in evaluating liver damage are levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) (Crawford and Iacobuzio, 2009). Elevated the serum levels of (ALT) and (ALP) confirmed the hyperglycemic liver dysfunction (Manna *et al.*, 2010). (Pinhas and Zeitler , 2007) demonstrated that serum alanine aminotransferase (ALT), which is an indication of liver damage, increased in about 20% of cases with type 2 diabetes mellitus in children and adolescents , in most cases this is lead to NAFLD.

(**Saligram *et al.*, 2012**) confirmed that there was a high incidence of elevated ALT in type 2 diabetes mellitus patients. Suggesting that start of liver abnormalities associated with disruption of blood sugar may precede the diagnosis of type 2 diabetes mellitus itself.

In the current study, the reduction of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in diabetic rats treated with Moringa seeds oil is consistent with results of ([Al-Said *et al.*, 2012](#)) who established that treatment with Moringa oleifera Lam seeds oil improved the healing from liver damage induced by CCl₄. The possible mechanism of Moringa oleifera Lam seeds oil in protection the liver may be due to probably free radical scavenging caused by the antioxidant compounds in the oil. Moreover, the results agree with the finding of (**Hamza, 2010**) who suggested that extract of Moringa seeds can treat the liver injury and fibrosis by a mechanism related to its antioxidant properties. ([Abd Eldaim *et al.*, 2017](#)) also reported similar results that an aqueous extract of Moringa oleifera leaves can be a powerful antioxidant and used as a protection agent for liver.

The findings in this study are agree to other studies, which demonstrated that treatment rats with extract of Moringa oleifera has protective effect of liver against (STZ) induced hepatotoxicity on serum levels of liver enzymes, which confirms its uses as antidiabetic and hepatoprotective agent ([Efiong *et al.*, 2013](#)). Moreover, (**Fakurazi *et al.*, 2008**) indicated that activity of Moringa oleifera extract in liver protection was observed in the significantly reduction of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in the pretreated (MO) groups compared with acetaminophen (APAP) group.

Furthermore, the study is consistent with (**Toppo *et al.*, 2015**) who concluded that supplementation of Moringa oleifera extract showed hepatoprotective effect against cadmium toxicity. There was significantly reduction in the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rats treated with cadmium and (MO).

Table (3): Effect of treating diabetic rats with *Moringa oleifera* seeds oil on serum lipid profile.

Parameters	G1(-ve) Nondiabetic rats	G2 Nondiabetic rats + <i>Moringa oleifera</i> seeds oil (1.8 mg/kg)	G3 (+ve) Diabetic rats	G4 Diabetic rats + <i>Moringa oleifera</i> seeds oil (1.8 mg/kg)
LDL (mg/dl)	85.3±0.2	79.9±0.6 ^c	105.8±0.4 ^{ab}	89.6±0.4 ^{bc}
HDL (mg/dl)	44.2±0.9	45.8±0.5 ^c	37.1±0.7 ^{ab}	40.8±0.3 ^{abc}
CHOL (mg/dl)	74.8±0.7	64.5±0.9 ^c	190±1.15 ^{ab}	86.5±0.9 ^{abc}

Values are represented as mean ± SD of 10 rats per group. ^(a) values are significant compared with nondiabetic rats. ^(b) values are significant compared with nondiabetic rats + moringa. ^(c) values are significant compared with diabetic rats.

In the present study, we obtained results showing that there were significantly increased in low-density lipoprotein (LDL) and total cholesterol(CHOL) levels in diabetic rats (G3) compared with non-diabetic rats (G1). However, treatment diabetic rats with *Moringa* seeds oil (G4) showed significantly reduction in (LDL) and (CHOL) compared with non-treated diabetic rats (G3). There was significant decreased in high-density lipoprotein (HDL) in diabetic rats (G3) compared with non-diabetic rats (G1). While, diabetic rats treated with *Moringa* seeds oil (G4) showed significantly elevated in (HDL) level in comparison with non-treated diabetic rats (G3).

Hyperlipidemia characterized by elevated levels of cholesterol, triglycerides, phospholipids and alteration in lipoproteins. It is one of diabetes complications and associated with hyperglycemia (Shew *et al.*, 2001). Lipid abnormalities related to diabetes are called dyslipidaemia instead of hyperlipidemia; the reason is that there may be changes in quantity and quality of the lipoproteins. Diabetes mellitus is a common

secondary reason of hyperlipidaemia, especially if uncontrolled glycaemia (**Jayarama et al., 2012**).

Lipid abnormalities which prevalent in diabetes mellitus are elevation of triglycerides, serum low-density lipoprotein (LDL), serum cholesterol and reduction of serum high-density lipoprotein (HDL) (**Bhambhani et al., 2015**). (**Ullasini and Priyanka, 2017**) confirmed that there is a significantly correlation between diabetes and lipid profile. Dyslipidaemia is highly spreading in diabetes mellitus patients and especially in those with uncontrolled diabetes.

The results are in agreement with (**Olatosin et al., 2018**) who reported that *Moringa oleifera* seed oil is helpful in regulating the metabolism of lipid and in prohibition hyperlipidemia complications. Moreover, (**Chumark et al., 2008**) indicated that *Moringa oleifera* have antioxidant and hypolipidaemic activities.

Furthermore, the results are consistent with (**Sugunabai et al., 2014**) who assessed the hypoglycemic and hypolipidemic effect of aqueous extract of *Moringa oleifera* leaves on diabetic and hyperlipidemic patients. Their results indicated that levels of glucose, total cholesterol and low-density lipoprotein were reduced and there was elevation in high-density lipoprotein in diabetic patients that treatment with *Moringa*. This effect could be because the presence of phytoconstituents. Also, the finding is similar to (**Oparinde et al., 2014**) who elucidated that dyslipidaemia was observed in alloxan induced diabetic rats. Administration of *Moringa oleifer* showed improvement in lipid parameters in diabetic rats than those without treatment.

Conclusions:

The study concluded that *Moringa* seeds oil can be protect the liver and reduce blood lipids in diabetes. The potential mechanism of *Moringa* seeds oil in protection the liver possibly resulting from ability of free radical scavenging by antioxidants in the oil.

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تأثير زيت بذور المورينجا اوليفيرا في وقاية الكبد و خفض دهون الدم
في الفئران المصابة بداء السكري

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الملخص العربي

أجريت هذه الدراسة لتقييم تأثير زيت بذور المورينجا اوليفيرا في حماية الكبد وخفض نسبة الدهون في الدم في الفئران المصابة بداء السكري. تم تقسيم أربعين من ذكور الفئران إلى أربع مجموعات. فئران غير مصابة بالسكر تغذت على الوجبة القياسية فقط (مج ١)، فئران غير مصابة بالسكر تغذت على الوجبة القياسية + زيت بذور المورينجا (١.٨ مجم / كجم) (مج ٢)، فئران مصابة بالسكر تغذت على الوجبة القياسية (مج ٣)، فئران مصابة بالسكر تغذت على الوجبة القياسية + زيت بذور المورينجا (١.٨ مجم / كجم) (مج ٤). أجريت الدراسة لمدة أربعة أسابيع. أظهرت النتائج ان وزن الكبد والوزن النسبي للكبد قد ازداد ازيداً ملحوظاً في الفئران المصابة بالسكر (مج ٣) مقارنة مع (مج ١) وانخفض بشكل كبير في الفئران المصابة بالسكر ومعالجة بزيت بذور المورينجا (مج ٤) مقارنة مع (مج ٣). أظهرت الفئران المصابة بالسكر ومعالجة بزيت بذور المورينجا (مج ٤) انخفاضاً ملحوظاً في مستوى الجلوكوز في الدم مقارنة مع الفئران المصابة بالسكر (مج ٣). كما أظهرت الفئران المصابة بالسكر (مج ٣) ارتفاعاً ملحوظاً في مستويات كلاً من (ALT)، (AST)، (ALP)، (LDL)، (CHOL) وانخفاضاً ملحوظاً في (HDL) مقارنة مع الفئران غير المصابة بالسكر (مج ١). الفئران المصابة بالسكر ومعالجة بزيت بذور المورينجا (مج ٤) أظهرت انخفاضاً ملحوظاً في مستويات إنزيمات الكبد وتحسناً ملحوظاً في مستويات دهون الدم مقارنة بالفئران المصابة بالسكر (مج ٣). استخلصت الدراسة أن زيت بذور المورينجا قادرًا على حماية الكبد وخفض نسبة دهون الدم في مرض السكر.

الكلمات المفتاحية: داء السكري، زيت بذور المورينجا، إنزيمات الكبد، دهون الدم