Potential activity of *Moringa Oleifera* leaf extract and some active ingredients against diabetes in rats

Fahmy T. Ali, Nahla S. Hassan* and Rehab R. Abdrabou

Abstract — *Moringa oleifera* has been regarded as a food substance since ancient times and has also been used for treatment of many diseases such as diabetes, hyperlipidemia and cardiovascular disease. The aim of this study is to evaluate the antidiabetic activity of *Moringa oleifera* leaf extract and three of its active ingredients (moringinine, quercetin and chlorogenic acid). Alcoholic extracts of *Moringa oleifera* leaf, moringinine, quercetin and chlorogenic acid; were tested against diabetic rats induced by alloxan. The aim was achieved via determination of glucose level, C-peptide, liver function tests, lipid profile and some oxidative stress markers. Pancreatic histopathology was also performed. Our results indicated that *Moringa oleifera* leaf extract counteracted the alloxan-induced diabetic effects in rats through normalization the elevated serum levels of glucose, triacylglycerol, total cholesterol, protein carbonyl content, malondi-aldehyde, total antioxidant capacity and C-peptide. Moreover, it restored the normal histological structure of the pancreas in diabetic rats. The result of our study suggests that alcoholic extract of *Moringa oleifera* leaf possess potent antidiabetic activity and also is a good source of natural antioxidants. Quercetin has the most potential antidiabetic activity in the extract, followed by chlorogenic acid and moringinine; the three compounds are responsible to a great extent for the antidiabetic activity of the whole extract.

Index Terms — *Moringa oleifera*; quercetin; moringinine; chlorogenic acid; anti-diabetic activity.

1 INTRODUCTION

*Moringa oleifera* L., is a tree that grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia. It is known as the drumstick tree based on the appearance of its immature seed pods, the horseradish tree based on the taste of ground root preparations, and the ben oil tree from seed-derived oils [1].

The Miracle Tree or *Moringa oleifera* Lam. (MO) is postulated to have the highest antioxidant content in food and also has a remarkable range of medicinal uses and high nutritional value. The leaves of this plant provide a rich source of carotenoids, vitamins, minerals, amino acids, alkaloids, and flavonoids and a rare combination of phenolic compounds, including zeatin, quercetin, kaempferol, apigenin, and many other phytoconstituents that offer essential and disease preventing nutrients to humans [2].

For centuries, *Moringa oleifera* has been used as a traditional medicinal source. Additionally, besides being edible, all the parts of the *Moringa* tree (e.g., pods, seeds, and leaves) have long been employed for the treatment of many diseases, and therefore, it was called a “miracle vegetable” [3].

Recently, various therapeutic effects of *Moringa oleifera* such as antimicrobial, anticancer, anti-inflammatory, antidiabetic and antioxidant effects have been investigated; however, most of these studies described only simple biological phenomena and their chemical compositions [4].

The flavonol quercetin is found at concentrations as high as 100 mg/100g of dried *Moringa oleifera* leaves [5], pre-dominantly as quercetin-3-O-β-d-glucoside also known as iso-querctrin or isotrifolin [6]. Quercetin is a potent antioxidant [7] with multiple therapeutic properties [8]. It has shown antidyslipidemic, hypotensive, and anti-diabetic effects in the obese Zucker rat model of metabolic syndrome [9].

Chlorogenic acid, which is an ester of dihydrocinnamic acid (caffeic acid) and quinic acid, is a major phenolic acid in *Moringa oleifera* leaves [10]. Chlorogenic acid can beneficially affect glucose metabolism. It has been shown to inhibit glucose-6-phosphate translocase in rat liver, reducing hepatic gluconeogenesis and glycogenolysis [11, 12].
The alkaloid moringinine was initially purified from Moringa oleifera root bark [13] and later chemically identified as benzyl amine [14]. It is also present in leaves. This substance was suspected to mediate the hypoglycemic effect of the plant.

Due to the increasing attention on natural products, such as those from plants, alcoholic extracts from Moringa oleifera leaves (MOL) have been prepared and their potential as new antidiabetic drug has been assessed in this study.

In this study, three of the best-characterized phytochemicals (quercetin, chlorogenic acid and moringinine) were used to evaluate the therapeutic efficacy in hyperglycemia, dyslipidemia, or related physiological conditions in induced diabetic rats.

2 MATERIALS AND METHODS

2.1 Preparation of Moringa oleifera leaf extract

Leaves of Moringa oleifera were collected from botanical garden of agriculture research center. Fresh matured leaves of Moringa oleifera were washed and shad dried. The air-dried leaves were made into a coarse powder. 200 mg of dried powdered leaves was macerated with petroleum ether to remove fatty substances, then the marc was further extracted with 2 litter ethanol using magnet stirrer for 24 hrs at room temperature. The extract was separated by filtration using filter paper no.1 the greenish extract was then evaporated in water bath at 50 °C to get thick mass, air dried and kept in deep freezer at -20 °C until use. The yield of dried extract obtained was 23.5% [15].

The yield of dried extract was calculated according to the following formula:

\[
\text{Yield (W/W %)} = \left( \frac{\text{weight of dried extract}}{\text{weight of starting material}} \right) \times 100
\]

2.2 Experimental animals

Eighty five Wistar male rats weighed about 185-200 g were purchased from Egyptian Organization for Biological Products and Vaccines (Helwaan Farm). The animals had free access to commercial pelleted diet and tap water before the start of the experiment (four per cage) and were provided a 1-week acclimatization period.

Diabetes was induced in fasting rats 12 h by a single intraperitoneal injection of freshly prepared alloxan (120 mg/kg body weight, dissolved in 0.9% saline,) [16]. After 48 h of alloxan treatment, rats with marked hyperglycemic (fasting blood glucose >200 mg/ dl) were selected and considered as diabetic, then divided equally into five groups each comprised 15 rats and 10 rats were run along experiment as control.

2.3 Experimental design

The various groups used in the experiment:

Group (1): Normal control rats, Group (2): Untreated diabetic group, rats supplied with alloxan only (120 mg/kg ), Group (3): Moringa leaf extract-treated group, diabetic rats treated with leaf extract at dose of (150 mg / Kg / day) for 21 days [17], Group (4): Moringinance-treated group, diabetic rats treated with moringinine at dose of (3600 µmole/Kg /day) for 21 days [18], Group (5): Quercetin treated group, diabetic rats treated with quercetin at dose of (30 mg/Kg/day) for 21 days [19] and Group (6): Chlorogenic acid treated group, diabetic rats treated with chlorogenic acid at dose of (10 mg/Kg/day) for 21 days [20].

At the end of the experiment animals, which survived (seventy four), were sacrificed by decapitation after overnight fasting and blood samples were collected in dry clean glass tube without additives to clot at 37 °C for 20 minutes, and then centrifuged at 3000 rpm for 10 minutes. The serum was then separated, divided into several aliquots and stored at -20 °C to be thawed once on demand. The sera of all studied groups were subjected to the following investigations, glucose [21], total cholesterol [22], triacylglycerol [23], LDL-c [24], HDL-c [25], alanine amino transferase (ALT) and aspartate amino transferase (AST) activities by Reitman and Frankel, [26]. Gama glutamyl transferase (GGT) activity [27].

Serum C-peptide was estimated by enzyme linked immunosorbant assay (ELISA) technique by commercial kit (RayBiotech, Inc.). Malondialdehyde; (MDA) by the thiobarbituric acid assay [28]. Total antioxidant capacity (TAC) according to Koracevic et al., [29]. Protein carbonyl content (PCC) by Levine et al., [30].

2.4 Pancreatic histopathology

Autopsy samples were taken from pancreas of different groups; fixed, washed and stained by hematoxylin and eosin stains for histopathological examination through the electric light microscope [31].

2.5 Statistical analysis

Statistical analysis was carried out by the aid of a digital computer, using Excel, and IBM SPSS Statistics version 21 program.

3 RESULTS

Results in Table 1 showed a highly significant increase in ALT, AST and GGT activities in diabetic untreated rats when compared with normal control. While, treatment with the extract and tested compounds to diabetic rats showed significant improve in the three enzymes as compared to diabetic group. Meanwhile; treatment with chlorogenic acid caused non-significant reduction in ALT activity.

Data presented in Fig. 1 showed marked increase in TAG, TC & LDLc and a significant
decrease in HDLc (diabetic untreated group). Meanwhile, in treatment groups there was a significant decrease in TAG, TC & LDLc and a significant elevation in HDLc compared to the diabetic rats.

As shown in figures 2 & 3, fasting blood glucose was significantly elevated; while C-peptide level was significantly decreased, in diabetic untreated rats. The treatment with the extract and its active ingredients significantly normalized the two parameters.

The antioxidant properties of the extract and the tested ingredients are obvious in figures 4, 5 and 6. In diabetic untreated group, there was a significant elevation in the level of malondialdehyde and the protein carbonyl content, however a significant reduction was observed in the total antioxidant capacity. These changes were nearly normalized in the treated groups.

The Receiver Operating Characteristic (ROC) curve and areas under the curves (AUC) for C-peptide and PCC are presented in figure 7 and table 2 showed, C-peptide yielded worse accuracy for diagnosing diabetes, while, PCC showed highest significant diagnostic performance (AUC 1.0 & cut off value 21.9). Moreover, MDA provided the highest diagnostic information in diabetic untreated group, with an AUC 1.0 and cut off value 16.25 (Table 3 & Fig.8).

Histopathological findings:
In the diabetic untreated group, there was congestion in the interlobular stromal blood vessels with degeneration and atrophy in the islands of Langerhans cells (fig.9, b). Treatment with the extract revealed no histopathological alteration in both endocrine islands of Langerhans cells as well as the acini of the exocrine portion (fig.9, c). In moringine treated group, mild degeneration and atrophy were noticed in the islands of Langerhans cells (fig.9, d).

The islands of Langerhans showed mild degeneration in some cells in group 5, which treated by quercetin (fig.9, e). There was no histopathological alteration recorded in chlorogenic treated group (fig.9, f).

4 DISCUSSION
Moringa has long been recognized in traditional medicine worldwide as having value both as a preventative and treatment agent of several health conditions, including the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal and haematological disorders [32].

Diabetes mellitus (DM) is one of the most important health problems worldwide. It is possibly the world’s fastest growing metabolic disorder, indicating high prevalence and mortality. Management of diabetes without any side effects is a challenge to medical communities, therefore herbal and natural products with anti-diabetic activity and fewer side effects are strongly needed [33].

In the present study, the pancreatic β cells were destroyed using alloxan (a toxic glucose analogue that accumulate in pancreatic beta cells via GLUT 2 glucose transporter). In the presence of thiols, especially glutathione (GSH), alloxan generates reactive oxygen species (ROS) in cyclic redox reactions. The eduction product of alloxan is dialuric acid [34].

Auto-oxidation of dialuric acid generates ROS, which are responsible for the death of the β cells. Alloxan also inhibits glucose-induced insulin secretion through its ability to inhibit the β cell glucose sensor, glucokinase.

Inappropriate activation of NFkB by ROS might start a cascade of events that result in an inflammatory and autoimmune response in pancreas, so the inhibition of NFkB activation by antioxidants could improve the severity of diabetes [35].

Treatment with the extract and its three ingredients ameliorate serum glucose concentration in alloxan diabetic rats, nearly to normal levels, besides elevating C-peptide concentrations which was reduced by alloxan administration.

The hypoglycemic effect of the extract was confirmed by Jaiswal et al. [36], using Streptozotocin (STZ) to induce diabetes in rats. Our data are in good agreement with other investigators [37]who stated that the positive effects of specific plant extracts on insulin activity suggest a possible role of these plants in improving glucose and insulin metabolism.

The anti-hyperglycemic effects of the extract and the tested ingredients are possibly linked to their antioxidant properties, which could counteract the toxic and pro-oxidant effects of alloxan.

Moyo et al., [38] reported a great amount of flavonoids, flavonols, phenols and proanthocyanidins in Moringa extract. These compounds have been reported to possess strong antioxidant and free radicals scavenging activity.

Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive anti-diabetic principles. Flavonoids are known to regenerate the damaged β cells in the alloxan diabetic rats. Phenolics were also found to be effective anti-hyperglycemic agents [39].

It is evident that increased hepatic glucose output in diabetes mellitus may be derived either from glycogenolysis or from gluconeogenesis or both [40]. This was confirmed by our results which showed a marked increase of the detected gluconeogenic serum enzymes; Alanine transaminase (ALT), Aspartate transaminase (AST) and gamma glutamyl transferase (GGT); compared to those of the nondiabetic ones. Our study demonstrated that, the treatment with
extract and its three ingredients resulted in the attenuation of liver injury induced by alloxan. These results are in accordance with those of Rawi et al., [40], who found that the decrease of transaminases activities with treatment may be attributed to improved liver function with the return of gluconeogenesis towards its normal rate.

In the present study, alloxan diabetic rats exhibited marked hyperglycemia, hypercholesterolemia with concomitant decrease in HDL cholesterol. Our results are in accordance with the findings of Mathe[41]; Ulicna et al., [42] and Wasan et al., [43] who recorded marked increases of serum triglycerides and cholesterol levels and abnormalities in lipoprotein levels in alloxan and Streptozotocin diabetic animals. These abnormalities certainly play a role in the increased risk for cardiovascular disease [44].

The abnormally high concentration of plasma lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the fat depot [45]. Treatment of alloxan diabetic rats with the extract and its three ingredients produced marked decrease of serum triglycerides and total cholesterol concentrations. The hypercholesterolemic action of the tested materials might be attributed to their ability to suppress cholesterol biosynthesis.

Sheikh et al., [46] reported that food supplementation of Moringa leaves provided protection against induced alteration of serum TC and HDLc.

Oxidative stress may constitute the key and common events in the pathogenesis of different diabetic complications [47]. Hypoinsulinemia in diabetes increases the activity of the enzyme fatty acyl coenzyme A oxidase which initiates β oxidation of fatty acids, resulting in lipid peroxidation [48]. In this study there was a significant elevation of plasma malondialdehyde (an indicator of lipid peroxidation) contents in diabetic rats. The extract, and also its ingredients, significantly reduced the lipid peroxidation product levels in diabetic rats. Total antioxidant capacity (TAC) reflects the ability to defend against free radical damage more precisely than measurement of individual plasma antioxidants, since TAC is a result of interactions among its various components. Our results indicated that TAC levels were markedly decreased in the diabetic group. It was observed that treatment with the extract, and also its ingredients, caused a significant increase in TAC.

These results were confirmed by Kirisattayakul et al., [49], who reported that Moringa oleifera decreases oxidative stress especially in cerebral cortex by decreasing MDA level and the elevation of antioxidant enzymes. Also the protein carbonyl content, which reflects the oxidative modified glycosylated protein, was significantly elevated in diabetic rats. That elevation was alleviated in the treated groups.

Leaves of Moringa oleifera contain alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, and tannins, which have therapeutic properties as antioxidants [50].

The histopathology results also confirmed our results, the whole extract and chlorogenic acid have restored the degeneration of the islands of Langerhans.

5 CONCLUSION

Finally, it is concluded that alcoholic extracts of Moringa oleifera leaves possess potent antidiabetic activity and also is a good source of natural antioxidant. The three tested active ingredients showed a potent antidiabetic activity. Quercetin has the most potential activity in the extract, followed by chlorogenic acid and moringinine, the three compounds are responsible to a great extent for the antidiabetic activity of the whole extract.

REFERENCES


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**TABLE 1**

**STATISTICS DESCRIPTIVE OF ALT, AST AND GGT IN ALL GROUPS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>N</th>
<th>Mean ±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALT (U/L)</strong></td>
<td>Control</td>
<td>10</td>
<td>46.67 ± 0.85*</td>
</tr>
<tr>
<td></td>
<td>Diabetic untreated</td>
<td>10</td>
<td>94.20 ± 1.90*</td>
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<tr>
<td></td>
<td>Diabetic treated with extract</td>
<td>15</td>
<td>64.73 ± 0.87 a,b</td>
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<tr>
<td></td>
<td>Diabetic treated with Moringinine</td>
<td>12</td>
<td>80.21 ± 1.94 a,b</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated with Quercetin</td>
<td>14</td>
<td>87.86 ± 1.47 a,b</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated with Chlorogenic acid</td>
<td>13</td>
<td>90.15 ± 1.78 a,b</td>
</tr>
<tr>
<td><strong>AST(U/L)</strong></td>
<td>Control</td>
<td>10</td>
<td>57.25 ± 0.66*</td>
</tr>
<tr>
<td></td>
<td>Diabetic untreated</td>
<td>10</td>
<td>111.80 ± 1.94*</td>
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<tr>
<td></td>
<td>Diabetic treated with extract</td>
<td>15</td>
<td>69.87 ± 1.59 a,b</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated with Moringinine</td>
<td>12</td>
<td>82.67 ± 1.96 a,b</td>
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<tr>
<td></td>
<td>Diabetic treated with Quercetin</td>
<td>14</td>
<td>86.71 ± 0.64 a,b</td>
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<tr>
<td></td>
<td>Diabetic treated with Chlorogenic acid</td>
<td>13</td>
<td>84.62 ± 1.62 a,b</td>
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<td><strong>GGT(U/L)</strong></td>
<td>Control</td>
<td>10</td>
<td>12.82 ± 0.27*</td>
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<tr>
<td></td>
<td>Diabetic untreated</td>
<td>10</td>
<td>34.02 ± 0.66*</td>
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<td>Diabetic treated with extract</td>
<td>15</td>
<td>18.01 ± 0.29 a,b</td>
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<td>12</td>
<td>23.13 ± 0.60 a,b</td>
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<td>Diabetic treated with Quercetin</td>
<td>14</td>
<td>23.14 ± 0.35 a,b</td>
</tr>
<tr>
<td></td>
<td>Diabetic treated with Chlorogenic acid</td>
<td>13</td>
<td>17.04 ± 0.51 a,b</td>
</tr>
</tbody>
</table>

*a: Significant difference at p< 0.05 compared with control group (G1).

b: Significant difference at p< 0.05 compared diabetic untreated animals (G 2).
**Fig. 1.** Lipid profile in all groups.


**Fig. 2.** Mean ±SE of fasting blood glucose in all groups.


**Fig. 3.** Mean ±SE of serum C-peptide in all groups.

Fig. 4. MDA level in all groups.

Fig. 5. TAC in all groups.

Fig. 6. Protein carbonyl content in all groups.

Fig. 7. Receiver operating characteristic (ROC) curves displaying the accuracy of C-peptide and PCC for diagnosing diabetic untreated groups.

**Table 2**

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area Under the Curve</th>
<th>Asymptotic Sig.</th>
<th>Cut off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-peptide (ng/ml)</td>
<td>0.000</td>
<td>&lt;0.001</td>
<td>5.5</td>
</tr>
<tr>
<td>PCC (nmol/ml)</td>
<td>1.000</td>
<td>&lt;0.001</td>
<td>21.9</td>
</tr>
</tbody>
</table>
Fig. 8. Receiver operating characteristic (ROC) curves displaying the accuracy of TAC and MDA for diagnosing diabetic untreated groups.

### Table 3

**Area under the curve and cut off value of TAC & MDA in diabetic untreated group**

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area Under the Curve</th>
<th>Asymptotic Sig.</th>
<th>Cut off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (mM)</td>
<td>0.000</td>
<td>&lt;0.001</td>
<td>2.95</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.000</td>
<td>&lt;0.001</td>
<td>16.25</td>
</tr>
</tbody>
</table>
Fig. 9. Hematoxylin and eosin-stained sections of rat pancreas. a: normal control b: diabetic untreated, c: diabetic treated with Extract, d: diabetic treated with Moringinine, e: diabetic treated with Quercetin, f: diabetic treated with Chlorogenic acid. (s): island of Langerhans cells, (a): the acini, (v): stromal blood vessels (ds) degeneration in island of Langerhans cells.