Effect of the ethanolic leaf extract of *Moringa oleifera* on insulin resistance in streptozotocin induced diabetic rats

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To cite this article: Anyanwu Anthony Chinedu., Salako Olanrewaju Alani., Adeyemi Olufunmi Olaide.. Effect of the Ethanolic Leaf Extract of *Moringa oleifera* on Insulin Resistance in Streptozotocin Induced Diabetic Rats. *Journal of Plant Sciences*. Special Issue: Pharmacological and Biological Investigation of Medicinal Plants. Vol. 2, No. 6-1, 2014, pp. 5-12. doi: 10.11648/j.jps.s.2014020601.12

Abstract: Insulin resistance plays a central role in the pathogenesis of type 2 diabetes mellitus. Ameliorating insulin resistance helps to improve glycaemic control and hence reduce the risk of development and progression of diabetic complications. The extract of *Moringa oleifera* has been shown to improve hyperglycaemia, however, little is known on its effect on insulin resistance and beta-cell function. The objective of this study was to determine the effect of the ethanolic leaf extract of *Moringa oleifera* on insulin resistance in high-fat diet and streptozotocin induced diabetic rats. Different doses (250 and 500 mg/kg) of the ethanolic leaf extract of *Moringa oleifera* were administered orally for two weeks, to high-fat diet and streptozotocin induced diabetic rats, and were compared with metformin 320 mg/kg and control group (distilled water 10 ml/kg) for the effect on fasting blood glucose, serum insulin and insulin resistance (HOMA). The extract of *Moringa oleifera* (at doses of 250 mg/kg and 500 mg/kg) significantly lowered the fasting blood glucose at days 7 and 14 compared to controls (p = 0.0021 and p = 0.0001 respectively). There was a significant increase in serum insulin level in the control group at days 7 and 14 compared to the groups treated with the extract (250 mg/kg, 500 mg/kg) and metformin (p<0.01, p <0.02 and p<0.01 respectively). The extract at both doses and metformin (320 mg/kg) induced a significant improvement in insulin resistance (HOMA-IR) on days 7 and 14 (p < 0.0001).

Keywords: Insulin Resistance, *Moringa oleifera*, Diabetes Mellitus, High-Fat Diet

1. Introduction

Insulin resistance plays a major pathophysiological role in type 2 diabetes and is tightly associated with major public health problems, including obesity, hypertension, coronary artery disease, dyslipidemias, and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome (DeFronzo et al., 1991, Petersen et al., 2004, Reaven et al., 2005).

Insulin resistance is typically defined as decreased sensitivity or responsiveness to metabolic actions of insulin, such as insulin-mediated glucose disposal and inhibition of hepatic glucose production. The concept of insulin resistance was proposed as early as 1936 (Himsworth, 1936) to describe diabetic patients requiring high doses of insulin.

The incidence of diabetes is on the rise and is estimated to be over 150 million worldwide (Wild et al., 2004). There is yet no effective cure for diabetes and the available drugs and insulin currently used in managing the disease are associated with several undesirable side effects (Piedrola et al., 2001; Yaryura-Tobias et al., 2001; Gandhipuram et al., 2006).

The word medicinal plant is any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs, preparation of surgical dressings (Sofowora, 1993), valuable for nutrition, for seasoning, dying and coloring of other materials (Kafaru, 1994).

Medicinal plants are widely used in management of diseases all over the world (Aliyu et al., 2007; Adewummi and Ojevole, 2004). Historically, the use of medicinal plants is as old as mankind and medicine. Different medicinal plants have different medicinal properties; no herb is found to be used for just one purpose (Kafaru, 1994). People all over the world have used herbs to cure and control different diseases that are peculiar to their sub-regions and Nigeria is no exception (Kafaru, 1994).

In Nigeria, several thousands of plant species have been
claimed to possess medicinal properties and employed in the treatment of many ailments (Okigbo and Mmeka, 2006). The leaf extract of the plant *Moringa oleifera* has been shown to have several beneficial metabolic effects in animal models, including blood glucose and lipid lowering effects (Ghasi et al., 2000; Tende et al., 2010); however, there are no reports on the effect of this plant extract on insulin resistance or beta-cell function.

1.1. Aim/Objectives

To determine the effect of the ethanolic leaf extract of *Moringa oleifera* on blood glucose levels and insulin resistance in high-fat diet and streptozotocin induced diabetic rats.

2. Materials and Methods

2.1. Apparatus

Glucometer and strips Accu-Check (Roche Diagnostics, Manheim, Germany), weighing scale (Mettle Pm 480 Delta ranged), oral canula, distilled water, plain and universal bottles, cages, 1 ml syringes and needles, Uniscope laboratory centrifuge, photomicroscope, insulin Elisa kit (ALPCO Diagnostics, U.S.A).

2.2. Drugs and Chemicals

Streptozotocin, STZ (Sigma Chemical Co., St. Louis, U.S.A), metformin (Biofem Pharmaceuticals Limited), 0.05M Citrate buffer, ethanolic leaf extract of *Moringa oleifera*.

2.3. Plant Materials

Fresh leaves of *Moringa oleifera* was purchased from herb sellers from the local Mushin market in Lagos, Nigeria in the month of June, 2012. Plant identification was done by Mr D.O. Oyebanji of the Department of Botany, University of Lagos, Lagos, Nigeria. The plant was assigned a voucher number LUH5010.

2.4. Plant Extract Preparation

The leaves of *Moringa oleifera* were collected and dried under shade and ground into powder. The powder (800g) was macerated in 70% ethanol at room temperature for 24 hours. It was then filtered using a filtered paper (Whatmann size no.1) and the filtrate evaporated to dryness in water bath at 60 °C. A brownish residue weighing 80.5 g was obtained and stored in air tight bottle in the refrigerator until when used. From this stock, a fresh solution of the extract (250 mg/ml and 500 mg/ml) dissolved in distilled water was prepared and used in the investigation.

2.5. Preparation of High-Fat Diet

A high-fat diet (HFD) was prepared by mixing calculated amounts of cornstarch, sucrose, casein, palmkernal cake, multivitamin tablets, soybean oil such that the final composition consisted of 41.2% fat (Ji Young Jung, 2011).

2.6. Animals, Induction of Diabetes and Diet

Male Sprague-Dawley rats were obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Ibadan, Lagos, Nigeria. They were kept in plastic cages under laboratory condition of temperature and humidity and placed on standard diet and allowed free access to water with 12 h light/dark cycle. For the experiments, a diabetic state was induced by feeding diet modified to contain 41.2% fat (HFD) for 2 weeks, followed by a single intraperitoneal injection of STZ (Sigma Chemical Co., St. Louis, MO, USA) at a low dose (40 mg/kg body weight, dissolved in 0.05 M citrate buffer, pH 4.5, immediately before use). One week after injection, fasting blood glucose (FBG) levels was determined from tail blood using an Accu-Check (Roche Diagnostics, Manheim, Germany). The rats with fasting blood glucose levels above 126 mg/dL were randomly divided into 4 groups (n = 5 for each group) as shown below (Ji Young Jung, 2011). Baseline fasting blood glucose and insulin resistance were determined and recorded.

The rats were assigned into 3 groups as follows:
- Group A were fed with a HFD and distilled water (diabetic control group).
- Group B were fed with a HFD and Metformin 320mg/kg/day orally,
- Group C was divided into 2 equal subgroups of 5 rats each (one group, C1 was fed with HFD and 250 mg/kg/day extract, and the other subgroup, C2 with HFD and 500 mg/kg/day extract) for 2 weeks.

The rats consumed diet and water ad libitum during the experimental period. Fasting blood glucose and insulin, Oral glucose tolerance tests (OGTTs) were then performed from the tail vein at the last week of the experimental period.

2.7. Oral Glucose Tolerance Test and Insulin Resistance

After overnight fasting for 8 to 12 h, blood samples for insulin were collected via ocular puncture into plain bottles after which they were centrifuged to separate the sera using Uniscope Laboratory Centrifuge (Model SM 902B, Surgifriend Medicals, England, U.K.) at 3500-4000 r.p.m. The animals were administered a glucose load (1 g/kg of body weight) dissolved in water by gavage. Blood glucose concentrations was determined from the tail vein by the glucose-oxidase principle (Beach and Turner, 1958), with Accu-Check (Roche Diagnostics, Manheim, Germany) at 0, 30, 60, 90, and 120 min.

Insulin resistance was derived using the Homeostasis model (Matthew et al., 1985; Hanley et al. 2002), by the following formula:

\[
\text{Insulin resistance} = \frac{\text{Fasting plasma insulin (µU/ml) \times Fasting blood glucose (mmol/L)}}{22.5} \text{ or } \frac{\text{Fasting insulin(µU/ml) \times Fasting blood glucose (mg/dL)}}{405}
\]

2.8. Determination of Blood Glucose and Insulin Levels

Blood samples were collected from the tail artery and orbital sinus of the rats. Determination of the blood glucose levels was done by the glucose-oxidase principle (Beach and
Turner, 1958). Accu-Check (Roche Diagnostics, Manheim, Germany) was used for the determination of the blood glucose levels of the animals and results expressed as mg/dl (Rheney and Kirk, 2000). Fasting insulin was determined using Elisa method (Insulin ELISA test kits, Mercordia diagnostic, USA).

2.9. Statistical Analysis

Blood glucose levels were expressed in mg/dL as mean±SEM and Insulin as mIU/L ±SEM. The data was statistically analyzed using ©2012 GraphPad Software, Inc. An interpolated was 13.18g/kg (appendix) (w/w) was obtained from the ethanolic leaf extraction.

The values of p<0.05 was taken as statistically significant. Using Elisa method (Insulin ELISA test kits, Mercor dia diagnostic, Germany) was used for the determination of the blood glucose levels of the animals and results expressed as mg/dl (Rheney and Kirk, 2000). Fasting insulin was determined using the method described by Miller and Tainter in 1944. The results are shown in Table 1.

3. Results

3.1. Physicochemical Properties of the Ethanolic Leaf Extract of Moringa Oleifera

A dark brownish extract, pH 6.5, having aromatic medicinal plants odour, coarse texture with a yield of 10.06% (w/w) was obtained from the ethanolic leaf extraction.

3.2. Acute Toxicity Test in Mice

Acute toxicity test was done to determine the median lethal dose of the ethanolic leaf extract of Moringa oleifera in mice. This was done using the method described by Miller and Tainter in 1944. The results are shown in Table 1.

Swiss albino mice, weighing 15 to 20 g, were used to determine the percentage death of animals 24 h after an oral dose. The animals were divided into five groups of 5 mice each. Ethanol extracts of M. oleifera were orally (through an oral cannula) given in single doses to the 5 groups, having 60 and 80% respectively. The percentage death were observed for 12 h and the number of animals that died in 24 h were recorded. The percentage deaths were 0, 20, 40, 60 and 80% respectively. The percentage death were converted to probit values which were plotted against the log dose of the extract. The value of the LD₅₀ obtained after interpolation was 13.18kg (appendix)

3.3. Anti-Diabetic Assessments

3.3.1. Effect of The Ethanolic Leaf Extract of Moringa Oleifera on Fasting Blood Glucose (FBG) on HFD/STZ Induced Diabetic Rats

Table 2 shows the effect of oral administration of distilled water (10 ml/kg/day), metformin 320 mg/kg/day and the leaf extract of Moringa oleifera (250mg/kg/day and 500 mg/kg/day) on fasting blood glucose in HFD/S induced diabetic rats at days 7 and 14. As shown, the extract induced a significant (p = 0.0021, p = 0.0001) hypoglycaemic effect both on days 7 and 14, for extract doses 250mg/kg and 500mg/kg respectively and a significant (p = 0.0017) hypoglycaemic effect for metformin 320 mg/kg, compared to control

Table 2. Effect of The Ethanolic Leaf Extract of Moringa Oleifera on Fasting Blood Glucose (FBG) in HFD/STZ Induced Diabetic Rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DOSE (mg/kg)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml/kg DW</td>
<td>198.3±40.6</td>
<td>186.8±28.3</td>
<td>188±23.1</td>
</tr>
<tr>
<td>% inhibition</td>
<td></td>
<td>5.8</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>320 mg/kg</td>
<td>210.4±49.7</td>
<td>75.8±7.5</td>
<td>56.8±0.6</td>
</tr>
<tr>
<td>% inhibition</td>
<td></td>
<td>63.9</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>M. oleifera</td>
<td>250 mg/kg</td>
<td>193.3±61.9</td>
<td>94±27.4</td>
<td>52±7.7</td>
</tr>
<tr>
<td>% inhibition</td>
<td></td>
<td>71.4</td>
<td>72.9</td>
<td></td>
</tr>
<tr>
<td>M. oleifera</td>
<td>500 mg/kg</td>
<td>187.5±29.8</td>
<td>76±6.5</td>
<td>55±7.2</td>
</tr>
<tr>
<td>% inhibition</td>
<td></td>
<td>59.5</td>
<td>70.3</td>
<td></td>
</tr>
</tbody>
</table>

(n=5). Values are expressed as mean±S.E.M. The extract (250 mg/kg and 500 mg/kg) induced a significant hypoglycaemia on days 7 and 14, when compared to control (p=0.0021, p=0.0001 respectively)

3.3.2. Effect of Oral Administration of Ethanolic Leaf Extract of Moringa Oleifera on Blood Glucose Level (OGTT) in HFD/STZ Induced Diabetic Rats

Table 3 shows the effect of oral administration of ethanolic leaf extract of Moringa oleifera and metformin on OGTT of HFD/S induced diabetic rats. The ethanolic leaf extract of Moringa oleifera (250 mg/kg and 500 mg/kg) and metformin (320 mg/kg) induced a significant (p=0.0003, p=0.0003 and p<0.0001) hyperglycaemia at 30 min. compared to control. There was no significant difference (p>0.05) in the change in glucose levels at 1 hr for both doses of the leaf extract.

Table 3. Effect of Oral Administration of Ethanolic Leaf Extract of Moringa Oleifera on Blood Glucose Level (OGTT) in HFD/STZ Induced Diabetic Rats.

<table>
<thead>
<tr>
<th>Blood glucose levels (mg/dl)</th>
<th>Treatment</th>
<th>0h</th>
<th>30min</th>
<th>1h</th>
<th>2h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water)</td>
<td>188±23.1</td>
<td>226±12.2</td>
<td>198±8.4</td>
<td>190±8.6</td>
<td>2</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-20.2</td>
<td>-5.3</td>
<td>-1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin 320 mg/kg</td>
<td>56.8±0.6</td>
<td>79.2±6.2</td>
<td>68.4±4.0</td>
<td>55±2.1</td>
<td>3</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-39.4</td>
<td>-20.4</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moringa oleifera 250mg/kg</td>
<td>52.2±7.7</td>
<td>71±4.2</td>
<td>55±2.0</td>
<td>52±2.2</td>
<td>4</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-36.0</td>
<td>-5.4</td>
<td>-0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moringa oleifera 500mg/kg</td>
<td>55.7±2.9</td>
<td>70±6.4</td>
<td>60±4.0</td>
<td>53±2.6</td>
<td>5</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-26.6</td>
<td>-7.7</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(n =5), values expressed as mean±S.E.M. A,b,c represent significant increase at p=0.0003, p=0.0003, p<0.0001 and p<0.0005. While d,e,f represent significant decrease at p=0.0001, P=0.0001 and p<0.00 at 2 hrs.

The leaf extract at doses of 250 mg/kg, 500 mg/kg and
Table 4. Effect of the Ethanolic Leaf Extract of Moringa Oleifera on Fasting Serum Insulin in HFD/STZ Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Fasting Serum Insulin (mU/dl)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Dose (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (DW)</td>
<td>0.99±0.18</td>
<td>2.7±0.92</td>
<td>13.9±7.6</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>320 mg/kg</td>
<td>1.55±0.4</td>
<td>1.07±0.3</td>
<td>0.84±0.1</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-172</td>
<td>-1304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. oleifera</td>
<td>250 mg/kg</td>
<td>0.95±0.4</td>
<td>1.33±0.5</td>
<td>1.22±0.1</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-30.9</td>
<td>45.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. oleifera</td>
<td>500 mg/kg</td>
<td>1.32±0.4</td>
<td>2.44±1.7</td>
<td>1.09±0.3</td>
</tr>
<tr>
<td>% inhibition</td>
<td>-84.9</td>
<td>17.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(=5) values expressed as mean±S.E.M. DW=distilled water. There is a significant increase in serum insulin level in the control group at days 7 and 14 compared to the group treated with the extract (250 mg/kg, 500 mg/kg) and metformin (p<0.01, p<0.02

3.3.4. Effect of the Ethanolic Leaf Extract of Moringa Oleifera on Body weight in HFD/STZ Induced Diabetic Rats

Figure 3 shows the effect of the ethanolic extract of Moringa oleifera on body weight in HFD/STZ induced diabetic rats.

Table 5. Effect of the Ethanolic Leaf Extract of Moringa Oleifera on Insulin Resistance (HOMA-IR) in HFD/STZ Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Homa-IR</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (DW)</td>
<td>0.48±0.02</td>
<td>1.25±0.06</td>
<td>6.43±0.43</td>
<td></td>
</tr>
<tr>
<td>% inhibition</td>
<td>-16.0</td>
<td>-1239</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>320 mg/kg</td>
<td>0.81±0.05</td>
<td>0.20±0.01</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>% inhibition</td>
<td>75.3</td>
<td>85.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. oleifera</td>
<td>250 mg/kg</td>
<td>0.45±0.06</td>
<td>0.31±0.03</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>% inhibition</td>
<td>31.1</td>
<td>64.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. oleifera</td>
<td>500 mg/kg</td>
<td>0.61±0.03</td>
<td>0.46±0.03</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>% inhibition</td>
<td>24.6</td>
<td>75.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(n=5), HOMA-IR, Homeostasis Model Assessment for Insulin Resistance. Values expressed as mean±S.E.M. The extract (250 mg/kg, 500 mg/kg) and metformin (320 mg/kg) induced a significant improvement in the insulin sensitivity on days 7 and 14 (p < 0.0001, p < 0.0001, p < 0.0001). and p<0.01 respectively)
4. Discussion

Type 2 diabetes mellitus (T2DM) is one of the world’s most common chronic diseases as changing modern lifestyles lead to reduced physical activity and increased obesity (Wild et al., 2004). Early phenomenon of T2DM is insulin insensitivity, which not only has negative metabolic consequences (Panda, 2007; Aslan, 2007) but also contributes subsequent pancreas β-cell exhaustion, resulting in the onset of clinical hyperglycemia (Stumvoll, 2007). Thus, understanding the regulation of the insulin response and identifying the related mechanisms are important to early treatment and prevention of T2DM.

A number of ways to improve insulin sensitivity have been proposed, because early treatment and prevention play a pivotal role in reducing the population burden of diabetes. Lifestyle changes such as losing weight, exercising, and watching the diet are often recommended, but have been difficult to maintain over a long term. Benefits of pharmaceutical factors to treat the disease aggressively early have been recommended, but medications may have unwanted side effects. Thus, there has been a growing interest in herbal remedies that can be introduced into the general population with the least side effects and the maximal preventive outcome (Matsuıı, 2006).

This study was designed to investigate the effects of the ethanolic leaf extract of *M. oleifera* on hyperglycemia, insulin resistance and beta-cell function in HFD/STZ-induced diabetic rats. To induce T2DM, a single low dose of STZ at 40 mg/kg body weight was injected combined with HFD. High doses of STZ (>45 mg/kg body weight) is well known to be taken by pancreatic β-cells via GLUT2 and to induce severe damages of pancreatic β-cells, mimicking type 1 diabetes mellitus (Rerup, 1970). On the contrary, the combination of HFD and low doses of STZ resulted in characteristic of T2DM; HFD induces insulin resistance and low doses of intraperitoneal STZ induce moderate impairment of insulin secretion (Reed et al., 2000; Srinivasan et al., 2005; Islam et al., 2008; Parveen et al., 2010).

The median lethal dose of the extract was determined according to the method described by Miller and Tainter (1944). After the oral administration of different doses (2, 5, 10, 20, 30 g/kg) of the extract, giving the percentage responses of 0, 20, 40, 60, 80% respectively (in probit), the LD₅₀ was interpolated as 13.18 g/kg (appendix), while the extract up to 2 g/kg dose given orally did not cause any death. Bruce (1987) described any chemical substance with LD₅₀ estimate greater than 2-5 g/kg/oral route as having a low toxicity and safe. Hence, the extract of *M. oleifera* can be considered relatively safe on acute oral exposure.

In this study, the anti-diabetic studies employed included a repeated extract treatment in HFD/STZ-induced diabetic rats. The oral administration of the ethanolic leaf extract of *M. oleifera* at doses of 250 and 500 mg/kg showed a significant improvement in the fasting blood glucose, glucose tolerance (OGTT) and insulin resistance of the HFD/STZ-induced diabetic rats on days 7 and 14 compared to the control (10 ml/kg distilled water) group. The extract at both doses and metformin also resulted in a weight loss on day 14 compared to the control group, which rather showed an increase in body weight. However, this weight loss was only statistically significant with the extract at a dose of 500 mg/kg.

The phytochemical screening result from the plant extract revealed the presence of secondary metabolites such as: flavonoids, terpenoids, saponin, and tannin. Studies have shown that terpenoids and flavonoids possess hypoglycaemic activities. The reported anti-inflammatory and antioxidant effects of flavonoids (Chumarketel., 2008; Vermaetal., 2009; Atawodi et al., 2010; Gupta et al., 2010; Sudha et al., 2010.) may also play a role in alleviating insulin resistance.

5. Conclusion

The result of this study revealed that the ethanolic leaf extract of *Moringa oleifera* has a potent anti-diabetic activity as it lowers blood glucose levels and improves insulin sensitivity and beta-cell function in diabetic rats. The possible mechanisms for its hypoglycaemic action may include: stimulation of pancreatic insulin secretion and/or ameliorating tissue insulin resistance. Further studies are needed in this area to ascertain the exact mechanism for the anti-diabetic effects of the plant extracts and the secondary metabolites involved.

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