Full Length Research Paper

Hepatoprotective and anti-diabetic effect of combined extracts of *Moringa oleifera* and *Vernonia amygdalina* in streptozotocin-induced diabetic albino Wistar rats


Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P. M. B. 1115, Calabar, Nigeria.

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This study was carried out to investigate the hepato-protective properties of combined extracts of *Moringa oleifera* (MO) and *Vernonia amygdalina* (VA) in streptozotocin (STZ) induced diabetic albino Wistar rats. The study design involved 42 rats weighing 120 to 180 g which were divided into 7 groups of 6 rats each. Groups 1 and 2 representing normal and diabetic control (NC and DC) received 0.5 ml of dimethylsulphoxide (DMSO). Group 3 received 5 IU/kg body weight insulin intraperitoneally and group 4 received 5 mg/kg body weight glibenclamide (GB) orally; groups 5 and 6 received 500 mg/kg body weight of MO and VA, respectively while group 7 the combined treatment group received 250 mg/kg body weight of each extract. After 28 days of treatment, the animals were anaesthetized and sacrificed to obtain blood by cardiac puncture. Serum was collected and assayed for alanine aminotransaminase (ALT), aspartate aminotransferases (AST) and alkaline phosphatase (ALP), total protein (TP) and albumin. The results showed that ALT, AST and ALP levels of the treatment groups decreased significantly (P<0.05) when compared with NC and DC, but were higher than reference drugs. The combined extract significantly (P<0.05) decreased the blood glucose sugar level, and this action compared favourably with the results obtained with the standard drugs. There was a significant increase (P<0.05) in TP and albumin levels of the treatment groups when compared with DC. Fasting blood glucose (FBG) showed significant decrease (P<0.05) in all treated groups compared to NC and DC. Moreover, the body weight change for the treatment groups showed a progressive decrease (P<0.05) except for groups treated with VA and MO when compared with NC. The results thus, show that single and combined extracts of MO and VA has hepatoprotective effects and may be safer in preventing diabetes induced damage to the liver.

**Key words:** Diabetes mellitus, liver enzymes, lipid profile, hepatoprotective, *Moringa oleifera*, *Vernonia amygdalina*.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of the endocrine system that precipitates disturbances in glucose, lipid and protein homoeostasis (Van den Berghe et al., 2006). The disease is found in all parts of the world and it is increasing rapidly worldwide. It is secondary to a deficiency of the number of pancreatic β-cells of the islets of Langerhans or resistance of tissue cells to insulin (Kelly and Fabtus, 1995; Lang et al., 2005). People suffering from diabetes cannot produce or properly use insulin, and so they persistently have high blood glucose. Diabetes is generally characterized by hyperglycaemia, glycosuria, polyuria, body weight loss, disability, coma and even death (Cockram et al., 1993). Type 2 diabetes is a non-insulin dependent diabetes mellitus, in which the body does not produce enough insulin.
or properly use it. It is the most common form of the disease, accounting for over 90% of the cases. Type 2 is nearing epidemic proportion as a result of an increased number of elderly people in the world, some of who are sarcopenic, and a greater prevalence of obesity and sedentary lifestyle. The cause of diabetes is a mystery, although both genetic and environmental factors such as lifestyle, obesity and lack of exercise appear to play a role (Jung et al., 2006). According to the World Health Organization (WHO) projection, the prevalence of diabetes is likely to increase by 35% by the year 2020. Currently, there are over 150 million diabetic patients world-wide and this is likely to increase to over 300 million by the year 2025. Statistical projection on India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025 making it as the country with the highest number of diabetics in the world (Tende et al., 2011). The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million by 2030 (Saxena and Kishore, 2004).

Plants are well known in traditional medicine for their hypoglycaemic effects and available literature indicates that there are more than 800 plant species showing hypoglycaemic activities in vivo (Maton et al., 1993). The maximum therapeutic and minimum side effects of herbal remedies have been verified in numerous scientific investigations. Presently, plant materials play major roles in primary health care as therapeutic remedies in many developing countries (Gagliano et al., 2007). This is the reason for which plant materials are continuously being scrutinized and investigated for their potential use as hypoglycaemic agents. Consequently, it has become imperative to investigate plants such as *Moringa oleifera* and *Vernonia amygdalina* which have been used by native populations as hypoglycaemic agents in a standardized experimentation. A number of investigations have shown that saponins, flavonoids and a host of other secondary plant metabolites including arginine and glutamic acid possess hypoglycemic effect in various animal models (Saxena and Kishore, 2004) and have been found to be hepatoprotective in diabetic animal experiments.

Currently, available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylurea, biguanides, α-glucosidase inhibitors, and glinides, which are used as monotherapies or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge (Wild et al., 2004). The liver is a vital organ in humans, which has a wide range of functions including detoxification of foreign substances in the body and protein synthesis. Other functions of the liver includes, building complex molecules from simple substances absorbed from the digestive tract, neutralization of toxins, manufacture of bile which aids fat digestion and removal of toxins through the bowels (Maton et al., 1993). However, the ability of the liver to perform these functions is often inhibited by numerous substances which the human body is exposed to on daily basis. These substances include drugs, medicines, alcohol and tobacco products all of which are injurious to the body and its organs (Gagliano et al., 2007). In the absence of reliable liver-protective drugs in medical practice, herbs have become reliable substitutes and have so far played significant role in the management of various liver disorders and the accompanying oxidative stress.

**MATERIALS AND METHODS**

**Collection of plants**

800 g of fresh leaves were harvested from the endocrine research farm of the University of Calabar, Nigeria.

**Chemicals and reagents**

All chemicals and reagents used in this study were obtained commercially and were all of analytical grade.

**Preparation of extract**

500 g each of the fresh leaves of *M. oleifera* and *V. amygdalina* were washed in clean water then macerated in 500 ml each of 80% ethanol and was each allowed to stand at room temperature for 48 h. The filtrates were evaporated in a rotary evaporator and allowed to concentrate on a water bath at 36°C. A greenish paste was obtained for each extract. The leaf extracts obtained were stored at <4°C until used.

**Experimental animals**

Forty two rats of the albino Wistar strain, weighing between 120 and 180 g, were obtained from the animal house of the Department of Biochemistry, University of Calabar, Nigeria. They were divided into 7 groups of 6 rats each and allowed to acclimatize for 7 days at room temperature (26±2°C), relative humidity (45 to 55%) and 12 hrs dark/light cycle. All the animals were fed with standard rat chow and water was allowed *ad libitum* under strict hygienic conditions.

**Induction of diabetes**

Streptozotocin (STZ) was prepared in citrate buffer (0.1 M, pH 4.5). 0.50 ml of the solution obtained was injected into each animal intraperitoneally at a concentration of 40 mg/kg body weight of rat. Type 1 diabetes was confirmed in fasting rats by determining the blood glucose levels after 72 h post diabetes induction. If the value obtained is more than 150 mg/100 ml, then diabetes has been successfully induced.

**Experimental design**

Forty two adult albino Wistar rats of both sexes, weighing 120 to
180 g were grouped into 7 groups of 6 rats each as follows: Group 1 served as the normal control (NC) group and were administered 0.5 ml dimethylsulphoxide (DMSO) orally; Group 2 was the diabetic control (DC) group and were administered 0.5 ml DMSO orally; Group 3 received 5 UI/kg body weight insulin (standard drug) intraperitoneally; Group 4 received 5 mg/kg body weight glibenclamide (GB, standard drug) orally; Group 5 received 500 mg/kg body weight of M. oleifera (MO) extract; Group 6 received 500 mg/kg body weight of V. amygdalina (VA) extract; Group 7 received 250 mg/kg body weight MO + 250 mg/kg body weight VA extracts.

Treatment was administered twice daily (12 hourly) for 28 days.

Table 1. Serum enzymes concentrations in treatment groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>58.44±20.34</td>
<td>13.11±4.21</td>
<td>7.12±2.61</td>
</tr>
<tr>
<td>DC</td>
<td>175.26±12.53 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.55±32.95 &lt;sup&gt;*,b&lt;/sup&gt;</td>
<td>12.61±0.18</td>
</tr>
<tr>
<td>INS</td>
<td>11.21±0.92 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.76±3.53 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.02±2.55</td>
</tr>
<tr>
<td>GB</td>
<td>14.81±7.09 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.64±2.56 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.84±0.67</td>
</tr>
<tr>
<td>VA</td>
<td>112.12±26.35 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.15±23.19 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.54±0.88</td>
</tr>
<tr>
<td>MO</td>
<td>62.19±24.64 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.88±3.70 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.89±1.55</td>
</tr>
<tr>
<td>MO+VA</td>
<td>45.93±14.10 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.14±3.98 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.48±3.19</td>
</tr>
</tbody>
</table>

*Significantly different from normal control (NC) at P<0.05. <sup>a</sup>Significantly different from diabetic control (DC) at P<0.05. <sup>b</sup>Significantly different from glibenclamide (GB) at P<0.05. <sup>*</sup>Significantly different from Vernonia amygdalina (VA) at P<0.05.

Values are Mean±SEM and n=3 determinations. INS: insulin.

Table 2. Serum proteins concentrations in treatment groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total proteins</th>
<th>Albumin</th>
<th>Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>5.99±0.61</td>
<td>4.55±0.31</td>
<td>1.44±0.53</td>
</tr>
<tr>
<td>DC</td>
<td>3.14±0.09</td>
<td>2.34±0.14</td>
<td>0.81±0.24</td>
</tr>
<tr>
<td>INS</td>
<td>3.76±0.69 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.80±0.51 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96±0.20 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GB</td>
<td>3.25±0.12 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.49±0.05 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76±0.16 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VA</td>
<td>4.82±0.93 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.75±0.65 &lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.89±0.28 &lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MO</td>
<td>3.92±0.07 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.35±0.03 *</td>
<td>1.58±0.04 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MV</td>
<td>5.23±0.10</td>
<td>4.46±0.10</td>
<td>0.77±0.20</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM and n=3 determinations. NC: Normal control; DC: diabetic control; GB: glibenclamide, MO: Moringa oleifera; VA: Vernonia amygdalina; INS: insulin.

**RESULTS**

Effects of treatment on liver enzyme markers

The activities of the three most prominent liver enzyme markers, alkaline phosphatase (ALP), alanine aminotransaminase (ALT), and aspartate aminotransaminase (AST) after treatment with plant extracts and standard drugs are shown in Table 1. The serum concentrations of the three liver marker enzymes reduced significantly (P<0.05) for all treatment groups when compared with the DC group. The combined extract treatment was more effective in reversing the enzyme activities when compared with the individual extract treatments. VA treated group significantly (P<0.05) reduced the activity of ALP (4.54±0.88 mg/dl), while the MO treated group was effective on AST (62.19±24.64 mg/dl), ALT (8.88±3.70 mg/dl) and ALP (7.89±1.55 mg/dl), respectively and compared well with the results obtained with the standard drugs.

**Effects of treatment on total protein**

Table 2 as well as Figure 1 showed the effect of treatments on total protein, albumin and globulin concentrations. Total serum protein for all treatment groups increased significantly (P<0.05) when compared with the DC group. The increase was observed with the results obtained with the standard drugs. The combined treatment group gave a more significant (P<0.05) increase in total protein albumin concentrations when compared with the single extract administered groups.

**Effects of treatment on body weight**

There was an initial reduction in body weights of experimental groups followed by a sudden gain in weights and the trend was the same with results obtained from the insulin, standard hypoglycemic drug (glibenclamide) and the combined extract treated groups (Table 3).

**Effects of treatment on fasting blood glucose level**

After the 28 days treatment, there was a significant (P<0.05) reduction in fasting blood glucose levels for the groups treated with plant extracts and the standard drug. The reduction was comparable with that obtained with the standard hypoglycaemic drug (glibenclamide) (Figure 1).

**DISCUSSION**

Type 1 diabetes was induced in albino Wistar rats using
Table 3. Change in body weights of the different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial weight</th>
<th>Day 6</th>
<th>Day 12</th>
<th>Day 18</th>
<th>Day 24</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>107.86±4.35</td>
<td>124.57±2.35</td>
<td>124.66±2.83</td>
<td>131.23±2.14</td>
<td>138.13±2.09</td>
<td>139.53±1.82</td>
</tr>
<tr>
<td>DC</td>
<td>140.96±13.0</td>
<td>142.20±9.31</td>
<td>133.54±12.78</td>
<td>139.78±10.43</td>
<td>145.70±15.00</td>
<td>141.72±12.78</td>
</tr>
<tr>
<td>INS</td>
<td>144.38±9.06</td>
<td>183.00±40.02</td>
<td>194.38±39.29</td>
<td>184.20±40.63</td>
<td>197.58±37.56</td>
<td>171.50±40.41</td>
</tr>
<tr>
<td>GB</td>
<td>156.80±0.89</td>
<td>149.65±2.29</td>
<td>164.55±3.31</td>
<td>171.80±11.30</td>
<td>153.83±8.73</td>
<td>157.83±5.31</td>
</tr>
<tr>
<td>VA</td>
<td>157.13±5.54</td>
<td>155.45±9.16</td>
<td>158.32±5.27</td>
<td>158.47±13.17</td>
<td>170.78±8.10</td>
<td>173.43±15.51</td>
</tr>
<tr>
<td>MO</td>
<td>141.45±5.05</td>
<td>142.65±4.95</td>
<td>137.05±0.65</td>
<td>142.75±23.75</td>
<td>160.60±8.80</td>
<td>162.45±11.85</td>
</tr>
<tr>
<td>VA+MO</td>
<td>159.30±12.28</td>
<td>161.40±13.05</td>
<td>148.85±5.10</td>
<td>149.23±6.54</td>
<td>144.02±8.80</td>
<td>146.60±11.12</td>
</tr>
</tbody>
</table>

Values are Mean±SEM and n=3 determinations. *Significantly different from NC at (P<0.05). NC: Normal control; DC: diabetic control; GB: glibenclamide, MO: Moringa oleifera; VA: Vernonia amygdalina; INS: insulin.

Figure 1. Initial and final blood glucose concentration of the different experimental groups. Values are Mean±SEM. *Significantly different from NC at P<0.05. aP<0.05 vs. DC; bP<0.05 vs. GB; cP<0.05 vs. INS; eP<0.05 vs. MO. NC: Normal control; DC: diabetic control; GB: glibenclamide, MO: Moringa oleifera; VA: Vernonia amygdalina; INS: insulin.

STZ. This resulted in the destruction of the majority of the β-cells of the islets of Langerhans and pancreatic dysfunction. Secondly, diabetes induction caused hepatotoxicity and oxidative stress to the liver, which was confirmed by the presence of oxidative marker enzymes in the blood. Diabetes inflamed lipid components in the plasma and electrolytes in the kidney, resulting in dyslipidemia and electrolyte disruption. Finally, the malfunctioning of the pancreas as a result of the diabetic condition led to an increase in blood glucose sugar and a general loss of weight in untreated diabetic animals.

The mechanism by which STZ induces diabetes is thought to be through its entry into the β-cells via a glucose transporter (GLUT2) where it causes alkylation of DNA molecule and its eventual damage. DNA damage in turn induces the activation of poly-ADP-ribosylation, a
process that is more important for the diabetogenicity of STZ than DNA damage itself. Poly-ADP-ribosylation leads to depletion of cellular nicotinamide adenine dinucleotide \((NAD^+)\) and adenosine triphosphate \((ATP)\). Enhanced ATP dephosphorylation after STZ treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals, hydrogen peroxide radicals and hydroxyl radicals \(in vivo\). STZ also liberates toxic amounts of nitric oxide into the body, and this inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, \(\beta\)-cells undergo destruction by necrosis (Szkudelski, 2001). In STZ-induced diabetic rats, the elevation in fasting blood glucose along with decrease in liver glycogen levels may be due to lower levels of plasma insulin (Maiti et al., 2004).

\(V.\ amygda\)lina have been shown to lower blood sugar with attendant weight benefits (Igile et al., 1995), and several studies have shown the benefits of this plant in the management of endocrine related diseases such as diabetic conditions (Atangwho et al., 2009). Studies have also confirmed the ethno-pharmacological use of \(M.\ oleifera\) in the management of diabetes related illnesses.

However, no studies have been carried out involving the administration of the combined leave extracts of these two plants that have shown so much promise in the alleviation of diabetic conditions in man. This study was carried out to compare the hepatoprotective effect, body weight management and blood glucose lowering efficacy of the combined extract of the two plants with the results of single plant extract administration.

The combined extract of the two plants effectively healed the liver and decreased the oxidative stress enzymes in the plasma. The combined extract caused weight gain in diabetic rats, increased albumin and protein levels in blood and significantly reversed diabetes in the rats by decreasing blood glucose sugar (Figure 1). These results are consistent with earlier results by Anwana and Garland (1991).

Both plant extracts when combined not only show their abilities in lowering the levels of oxidative stress enzymes \(\text{ALT, AST and ALP}\) in serum, but similar with the standard anti-diabetic drugs \(\text{glibenclamide and insulin}\). The observed significant increase in the activity of AST alone confirms it is a more reliable marker of liver integrity than AST.

Albumin and globulin are important in the transport of hormones synthesized in the liver and serve as indicators of the immune status especially in disease states. Hepatotoxicity and general oxidative stress caused by diabetic conditions, directly affect the integrity and functioning of these two biomolecules. The results in this study showed that, combined ethanolic leave extract of both plants showed a greater ability in synthesizing albumin and globulin when compared with the standard anti-diabetic drugs. These findings are promising, because it gives hope for the better management of diabetes mellitus over the conventional medications due to the fact that it is safe, cheap and readily available.

Diabetes mellitus has become a growing problem in the world today (Piyush et al., 2006). It is associated with genetic factors, environmental factors and general lifestyle. In most cases, it is said to have its origin from obesity, which directly predisposes the diabetic condition. At the present rate of its increase, it may turn out to be one of the world's most common diseases and biggest public health burdens, with an estimated minimum of half-a-billion cases over the next decade (Joseph and Jini, 2011; Diamond, 2003). Nigeria with an estimated population of nearly 200 million people and its pattern of diets and lifestyle is already an obsessed and diabetic society. This astronomical increase in the prevalence of diabetes has made diabetes a major public health challenge for Nigeria and many other countries. The Nigerian population benefits from the indigenous ethnopharmacological flora in the management of diabetes and other metabolic diseases. The study showed that the combined administration of \(V.\ amygda\)lina and \(M.\ oleifera\) produced weight gain, hypoglycaemia and hepatoprotection in experimentally induced type 1 diabetic albino Wistar rats. The findings in this study are similar to other studies of researchers, who had variously demonstrated that the extract from \(V.\ amygda\)lina possesses antidiabetic properties (Minari, 2012; Akah and Okafor, 1992a,b; Sabu and Kutan, 1982). \(M.\ oleifera\) have also been shown to possess glucose lowering effect in STZ-induced diabetic rats by possibly stimulating the \(B\)-cells of the islets of Langerhans or due to its insulin-like activity (Tende et al., 2011). \(M.\ oleifera\) and \(V.\ amygda\)lina leaves extract have also shown some protective effect on the liver from other studies (Bura\-imoh et al., 2010; Ojiako and Nwanjo, 2006). The aqueous leaf extract of \(V.\ amygda\)lina may have exhibited hepato-protective activity due to its antioxidant property and its flavonoid content (Igile et al., 1994), and also may be as a result of the sesquiterpene lactones and saponins constituents.

The administration of combined leave extracts of both plants showed a potential effect which may be due to a synergy between the bioactive secondary compounds from these two plants. This agrees with another study which showed that there appears to be a complement of bioactive principles in the leaves of these plants, and this may account for their hypoglycemic action (Atangwho et al., 2009).

**Conclusion**

The combined herbal treatment has demonstrated liver protective effect against STZ-induced hepatotoxicity on serum levels of liver enzymes and total proteins, thus confirming their uses as hepatoprotective and anti-diabetic agents. This study showed that combined extract
of *M. oleifera* and *V. amygdalina* can protect against STZ-induced toxicities. The present findings provide scientific evidence that 100 mg/kg body weight of combined extract of *M. oleifera* and *V. amygdalina* is nearly as potent as 1 unit of insulin in reversing oxidative stress and hyperglycemia in diabetic rats.

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