Attenuation of alloxan-induced disturbance of glucose metabolism, liver function and antioxidant status by methanol leaf extract of *Synsepalum dulcificum*

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**ABSTRACT:** The effect of a methanol leaf extract of *Synsepalum dulcificum* on biochemical indices of hyperglycemia, oxidative stress and liver function in alloxan-induced diabetic rats was examined. Diabetes was induced by the administration of 125 mg/kg alloxan to Wistar rats. Animals with confirmed hyperglycemia were treated with 25 mg/kg MSD, 50 mg/kg MSD or 2 mg/kg glibenclamide and sacrificed after 14 days of treatment. Serum glucose concentration, activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) alkaline phosphatase (ALP) and superoxide dismutase (SOD) and levels of reduced glutathione (GSH), malondialdehyde and total protein were evaluated. Animals in the diabetic control group had a significantly higher glucose level compared with the normal control, extract- and glibenclamide-treated diabetic animals (P<0.05). The percentage weight loss in extract and glibenclamide treated diabetic animals was lower compared to the value for diabetic controls. Moreover, the diabetic control group had significantly higher activities of AST, ALT, and ALP compared with the normal control, extract and glibenclamide treated groups (P<0.05). Malondialdehyde level in the diabetic control animals was higher compared to normal control animals and animals in the treated groups (P<0.05) while the reverse pattern was observed for total protein level, GSH concentration and SOD activity. The results suggest that methanolic extract of *Synsepalum dulcificum* may be useful in combating diabetes mellitus.

**KEYWORDS:** free radicals, hyperglycemia, malondialdehyde, *Synsepalum dulcificum.*
INTRODUCTION

Diabetes mellitus is a multifactorial, multisystemic endocrine disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2012). Persistent hyperglycaemia and the development of diabetes-specific microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral arterial disease) complications are the main characteristics of all forms of diabetes mellitus. The importance of protecting the body against persistent elevation of blood glucose cannot be overemphasized because its direct and indirect effects on the human vascular system are the major causes of morbidity and mortality in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) (Fowler, 2008). Apart from hyperglycaemia diabetes mellitus is characterized by elevated level of oxidative stress indices e.g. lipid peroxidation, decreased level of antioxidant defenses and dyslipidemia (Amos et al., 1993). The integrity of the liver is compromised in diabetes mellitus and this is reflected in disturbances in glucose metabolism and the levels or activities of biomarkers of liver function.

The International Diabetes Federation (IDF) estimated that the global burden of diabetes was 366 million in 2011 and it would rise to 552 million by 2030 (Vaz and Patnaik, 2012). It has also been reported that the world’s prevalence of diabetes among adults (aged 20–79 years) was 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7%, and 439 million adults by 2030 and between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw et al., 2010).

The orthodox approach to the management of diabetes mellitus has always included lifestyle modification and dietary therapy, administration of oral antidiabetic drugs, and insulin therapy (Akinmoladun et al., 2014). Despite the successes recorded with these treatment modalities some undesirable side effects are also experienced. For instance, up to 2.5% and 17.5% of sulfonylurea (SU)-treated patients experience major and minor hypoglycaemia respectively, while gastrointestinal (GI) problems affect up to 63% of metformin, and 30% of acarbose treated patients (Ho et al., 2006). Other side effects are weight gain associated with sulfonylureas; weight gain, GI disturbances and liver injury associated with thiazolidinediones and meglitinides; flatulence, diarrhea and abdominal bloating associated with alpha-glucosidase inhibitors (Bastaki, 2005). These issues have made the search for newer and better treatment modalities with fewer side effects for diabetes mellitus fundamental and there has been an increasing focus on medicinal plants with antioxidant and pharmacologically active polyphenolic and other phytochemical compounds (Farnsworth et al., 1985; Halliwell, 1997; Alarcon-Aguilara et al., 1998; Loew and Kaszkin, 2002; Naik et al., 2006; Sati et al., 2010; Aliyu et al., 2011). Moreover, most of the current medications in use are distillations, combinations, reproductions or variations of substances that are abundantly found in nature (Senthil et al., 2006). Therefore, plants have the potential to provide new treatments for diabetes because they are cheap and more easily accessible, less toxic and in some cases more efficacious than synthetic drugs.

**Synsepalum dulcificum** Daniell (Sapotaceae) is an evergreen shrub native to tropical West Africa, and the fruits have the property of modifying sour taste into sweet taste remarkably (Chen et al., 2010). The fruit has been reported to improve insulin resistance induced by fructose-rich chow in rats (Chen et al., 2006). However, to the best of our knowledge the antidiabetic potential of the leaves has not been evaluated and this is the focus of the present study.

MATERIALS AND METHODS

**Plant material**

*Synsepalum dulcificum* leaves were obtained from a farmland at Iseyin in Oyo State, Nigeria. Identification was carried out at the Botany Department, University of Ibadan Oyo State, Nigeria. The leaves were air-dried and pulverized. The pulverized sample (700 g) was extracted in 80% methanol by maceration for 72 hours. The methanol extract of *Synsepalum dulcificum* (MSD) was concentrated in a rotary evaporator, lyophilized and preserved for further use.

**Chemicals**

Alloxan was obtained from Sigma-Aldrich Co. (St Louis MO, USA). All other chemicals and reagents used were of analytical grade. Assay kits for glucose, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were obtained from Randox Laboratories Ltd., Antrim, UK.
**Animals**

Forty two Wistar rats weighing 170-190 g were obtained from the animal house of the Department of Chemical Sciences, Afe Babalola University, Ado Ekiti, Nigeria. The rats were acclimatized for two weeks before the commencement of the experiments and fed on standard rodent diet and water ad libitum. All experiments were approved by the ethics committee of the University and were therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**Induction of Diabetes**

Animals were randomly distributed into seven groups with six rats per treatment group. Group 1 was fed standard diet and water ad libitum and served as the negative control. The group received physiological saline throughout the period of the experiment. Group 2 was administered a single intraperitoneal dose of 125 mg/kg of alloxan monohydrate. Groups 3 and 4 received only 25 mg/kg and 50 mg/kg MSD, respectively. Groups 5, 6 and 7 were treated as in group 2 and in addition received 25 mg/kg MSD, 50 mg/kg MSD and 2 mg/kg glibenclamide, respectively. The administered doses were based on pilot studies (data not shown). Induction of diabetes mellitus was confirmed after the third day of alloxan treatment using ACCU-CHEK™ test strips and blood glucose meter according to the instructions of the manufacturer.

Rats with fasting blood glucose level ≥ 250 mg/dl were selected for the study. Treatment with MSD and glibenclamide commenced only after the confirmation of diabetes. After 14 days of treatment, animals were fasted for 12 h, and sacrificed by anaesthesia. Blood was collected by cardiac puncture into sample tubes, allowed to clot and centrifuged at 3000 rpm for 10 min to obtain sera used for biochemical analyses.

**Biochemical assays**

Glucose and total protein concentration and the activities of ALT, AST and ALP were estimated using assay kits. SOD activity was determined by the method of Misra and Fridovich (Mishra and Fridovich, 1992), reduced glutathione (GSH) was determined by the method described by Beutler (Beutler et al., 1963). MDA concentration was assayed using the method of Gerard-Monnier (Gerard-Monnier, 1998).

**Statistical analysis**

The results were expressed as mean ± SD. Differences between means were determined by one-way analysis of variance (ANOVA) followed by Duncan’s test. P<0.05 was considered statistically significant.

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**Table 1: Effect of methanol extract of *Synsepalum dulcificum* leaves (MSD) on blood glucose level (mg/dl) of alloxan-induced diabetic rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.30 ± 2.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.86 ± 20.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.20 ± 2.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alloxan</td>
<td>81.75 ± 1.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>393.75 ± 8.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>293.50 ± 7.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 mg/kg MSD</td>
<td>84.28 ± 1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.46 ± 1.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.17 ± 2.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 mg/kg MSD</td>
<td>86.39 ± 2.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85.83 ± 3.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.28 ± 3.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alloxan + 25 mg/kg MSD</td>
<td>84.50 ± 4.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>281.25 ± 7.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.00 ± 3.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alloxan + 50 mg/kg MSD</td>
<td>81.00 ± 3.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>372.75 ± 6.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>112.00 ± 4.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alloxan + 2 mg/kg Glibenclamide</td>
<td>78.00 ± 4.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>343.75 ± 6.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.50 ± 2.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different. Each value is a mean of six determination ± SD.
RESULTS

The control, extract or glibenclamide treated groups showed a significantly (p<0.05) lower serum glucose level than the diabetic control group after 14 days of treatment. A weight loss of 35.38% was observed in the diabetic control group while the control and extract or glibenclamide treated groups gained weight in the course of the study. The activities of AST, ALT and ALP in the serum of the diabetic control group were significantly higher than those of the extract or glibenclamide treated groups.

The diabetic control group had a significantly (p<0.05) lower SOD activity and total protein and GSH levels than all the other groups in the study, while the MDA levels observed in the diabetic control group was significantly (p<0.05) higher than all the other groups.

DISCUSSION

The deleterious effects of alloxan on the insulin secreting cells of the pancreas is well documented (Szkudelski, 2001). Alloxan prompts diabetes by destroying the insulin secreting cells of the pancreas resulting in hypoinsulinemia and hyperglycemia (Szkudelski, 2001). This was confirmed in the present study by the increased serum glucose concentration of the diabetic control group compared with the normal control (p<0.05) (Fig 1).

The results presented here showed further that the methanol leaf extract of *Synsepalum dulcificum* at both doses employed demonstrated remarkable antihyperglycemic effect as glucose levels in the MSD treated groups were brought to level obtained in normal control animals following 14 days of treatment (p>0.05).

Decrease in body weight is considered a marker for the development of diabetes due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis (Salau et al., 2003). Increased catabolic reaction leading to muscle wasting can also be the cause of the reduced body weight (Hakim et al., 1997). In this study, the body weights of the untreated diabetic animals were found to progressively decline significantly (p<0.05) through the course of the study when compared with the animals in the control, glibenclamide and extract treated groups. On the contrary, the average body weights of the extract treated animals were found to increase by the end of the study, an indication of the improvement of catabolic reactions and the associated body wasting characteristic of diabetes. This result is in agreement with earlier studies that showed a progressive reduction in body weight of both alloxan and streptozotocin induced diabetic animals (Sikarwar and Patil, 2010a; Sikarwar and Patil, 2010b). The results indicate that the plant extract probably normalized glucose metabolism.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Weight Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>178.98±17.24a</td>
<td>201.77±13.75c</td>
<td>12.73</td>
</tr>
<tr>
<td>Alloxan</td>
<td>190.80±5.45b</td>
<td>123.29±4.29a</td>
<td>-35.38</td>
</tr>
<tr>
<td>25 mg/kg MSD</td>
<td>172.67±10.20a</td>
<td>198.23±10.12c</td>
<td>14.80</td>
</tr>
<tr>
<td>50 mg/kg MSD</td>
<td>170.56±9.24a</td>
<td>203.39±11.23c</td>
<td>19.24</td>
</tr>
<tr>
<td>Alloxan + 25 mg/kg MSD</td>
<td>161.73±6.38a</td>
<td>168.94±6.66b</td>
<td>4.46</td>
</tr>
<tr>
<td>Alloxan + 50 mg/kg MSD</td>
<td>160.55±11.63a</td>
<td>166.55±11.61bc</td>
<td>3.74</td>
</tr>
<tr>
<td>Alloxan + 2 mg/kg Glibenclamide</td>
<td>167.90±15.27a</td>
<td>178.51±15.51c</td>
<td>6.32</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different. Each value is a mean of six determination ± SD.
Table 3: Effect of methanol extract of *Synsepalum dulcificum* leaves (MSD) on serum AST (IU/L), ALT (IU/L) and ALP (μmol/L) activities of alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.02±1.02a</td>
<td>21.06±2.03a</td>
<td>13.23±4.11b</td>
</tr>
<tr>
<td>Alloxan</td>
<td>68.38±10.03c</td>
<td>35.82±2.72b</td>
<td>31.23±2.29c</td>
</tr>
<tr>
<td>25 mg/kg MSD</td>
<td>18.22±2.23a</td>
<td>16.31±0.17a</td>
<td>23.41±7.33b</td>
</tr>
<tr>
<td>50 mg/kg MSD</td>
<td>28.17±1.33a</td>
<td>22.23±1.25a</td>
<td>17.26±4.82b</td>
</tr>
<tr>
<td>Alloxan + 25 mg/kg MSD</td>
<td>27.45±5.81a</td>
<td>18.56±2.19a</td>
<td>12.22±2.26a</td>
</tr>
<tr>
<td>Alloxan + 50 mg/kg MSD</td>
<td>51.26±6.21b</td>
<td>13.17±0.06c</td>
<td>20.01±3.11b</td>
</tr>
<tr>
<td>Alloxan + 2 mg/kg Glibenclamide</td>
<td>27.64±4.17a</td>
<td>20.08±1.12a</td>
<td>12.54±2.84a</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different. Each values is a mean of six determination ± SD. AST, Aspartate Transaminase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase.

Table 4: Effect of methanol extract of *Synsepalum dulcificum* leaves (MSD) on serum TP (g/dl), MDA (×10⁶ nmol/ml), SOD (Units/mg of protein) and GSH (μg/mg of protein) on alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TP</th>
<th>MDA</th>
<th>SOD</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.02±2.71a</td>
<td>3.15±0.59a</td>
<td>72.31±9.24a</td>
<td>232.57±0.53b</td>
</tr>
<tr>
<td>Alloxan</td>
<td>1.42±0.08a</td>
<td>7.78±0.27a</td>
<td>20.88±0.25b</td>
<td>121.10±0.81a</td>
</tr>
<tr>
<td>25 mg/kg MSD</td>
<td>6.62±2.38c</td>
<td>3.91±0.73b</td>
<td>67.70±9.19b</td>
<td>232.10±0.66b</td>
</tr>
<tr>
<td>50 mg/kg MSD</td>
<td>3.09±0.25c</td>
<td>5.47±0.70b</td>
<td>87.71±3.77c</td>
<td>232.82±0.95b</td>
</tr>
<tr>
<td>Alloxan + 25 mg/kg MSD</td>
<td>6.04±0.56c</td>
<td>3.04±0.50b</td>
<td>76.16±12.02b</td>
<td>231.83±0.50b</td>
</tr>
<tr>
<td>Alloxan + 50 mg/kg MSD</td>
<td>3.09±0.41c</td>
<td>2.98±0.04b</td>
<td>94.62±1.47c</td>
<td>230.53±0.85b</td>
</tr>
<tr>
<td>Alloxan + 2 mg/kg Glibenclamide</td>
<td>11.78±0.31b</td>
<td>3.08±0.30b</td>
<td>73.38±1.88b</td>
<td>231.52±1.19b</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different. Each values is a mean of six determination ± SD. TP, Total Protein; MDA, Malondialdehyde; SOD, Superoxide Dismutase; GSH, Glutathione.
leading to enhanced body weight in the rats. A similar observation was reported by Ravi et al. (1997) and Hakim et al. (1997).

The transaminases-AST and ALT are important biomarker enzymes used to predict possible toxicity and damage to the liver (Rahman, 2001). Moreover, ALP is a liver biomarker enzyme used to assess the integrity of plasma membrane and endoplasmic reticulum (Akanji, 1993). Elevation in the activities of serum ALT, AST and ALP are suggestive of damage to the liver cells (Wolf et al., 1972) as well as predictors of diabetes (Pradeep et al., 2013).

The observed lower activities of AST, ALT and ALP in extract treated animals may imply stabilization of plasma membrane as well as repair of damaged hepatic tissue that can be considered as an expression of the functional improvement of the hepatocytes (Nirala and Bhadauria, 2008). Similar observation have been reported in other studies (Ravi et al., 2004).

Experimentally induced diabetes in rat model caused alterations of amino acid metabolism, which may be attributed to increased muscle proteolysis, reduced protein synthesis which is an energy dependent process in the liver, and stimulated hepatic gluconeogenesis utilizing gluconeogenic amino acids (Fando et al., 1985). The results shown in Table 5 showed a marked reduction in the total serum protein in the animals in diabetic control group as compared with the normal control group. However a significant improvement in the serum protein levels was observed in the animals treated with extracts and the standard drug. This suggests that the extract has the potential to ameliorate the alterations in amino acid metabolism associated with diabetes mellitus.

The induction of diabetes by alloxan produced a significant decrease in antioxidant capacity. This finding is in accord with that of Jackson et al. (2007) that the antioxidant capacity decreased in alloxan induced diabetic rats compared to normal control. Malondialdehyde (MDA) is a degradative product of peroxidation of polyunsaturated fatty acids in the cell membrane and is a reliable marker of oxidative stress which has been reported as one of the underlying causes of diabetes mellitus (Ceriello, 2000; Akinosun and Bolajoko, 2007).

The result presented in Table 6 shows that the MDA levels in the serum of diabetic untreated animals was significantly (p<0.05) higher than the values observed in the control group. MDA levels in the normal control, glibenclamide treated and extract treated groups were also not significantly different (p<0.05). The reversal of alloxan provoked increase in MDA level revealed the antioxidant potential of the extract. This is supported by the results for the other oxidative stress markers. The tripeptide thiol, GSH, is highly important in antioxidant and cellular defense networks in organisms. It serves as a hub for the maintenance of proper redox state (Ghanta and Chattopadhyay, 2011). The enzyme, SOD is the first line of antioxidant defense against ROS generated by respiration and, among eukaryotic organisms, it is the only enzyme that can detoxify superoxide. SOD acts by converting superoxide to hydrogen peroxide, which can subsequently be converted to water by catalase or peroxiredoxin (Van Raamsdonk and Hekimi, 2012). The reversal of alloxan-induced alterations to these critical oxidative stress biomarkers by MSD showed its remarkable ability to mitigate oxidative stress.

**Conclusion**

The findings from this work leads to the conclusion that Synsepalum dulcificum leaf extract, and not just the fruit extract as shown by previous workers, possess remarkable antidiabetic potential. The antidiabetic property of Synsepalum dulcificum leaf extract against alloxan induced diabetes in this study involved reversal of oxidative stress condition and improvement of glucose metabolizing capacity of the liver.

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**REFERENCES**


