Comparative Evaluation of the Protective Effect of the Ethanolic and Methanolic Leaf Extracts of Sida acuta Against Hyperglycaemia and Alterations of Biochemical and Haematological Indices in Alloxan Diabetic Rats

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Abstract: The antioxidant property of Sida acuta was recently demonstrated, in vitro. We proposed that this property could confer some protective benefit in alloxan model of diabetes mellitus in rats and compared the efficacy of the methanolic and ethanolic leaf extracts of the plant in this study. Diabetes was induced with alloxan monohydrate (150 mg kg⁻¹, intraperitoneally). Diabetic rats were divided into six groups of 7 rats/group and treated with either ethanolic extract of Sida acuta (EESA), methanolic extract of Sida acuta (MESA, 200 and 400 mg kg⁻¹) or glibenclamide (200 mg kg⁻¹) orally for three days. Diabetic and normoglycaemic control received normal saline (10 mL kg⁻¹). Animals were sacrificed by cervical dislocation 24 h after last administration. The EESA and MESA exhibited similar hypoglycaemic activity at 200 mg kg⁻¹. The EESA (200 mg kg⁻¹) significantly (p<0.01) lowered malondialdehyde and increased glutathione (GSH) and Uric Acid (UA) at 400 mg kg⁻¹. The MESA increased GSH at both doses, produced mild decrease in malondialdehyde and increased UA at 200 mg kg⁻¹ (p>0.05). Both extracts significantly reduced plasma total cholesterol (p<0.05) and triglycerides (p<0.001). The hypoglycaemic and hypolipidaemic effects of EESA and MESA were comparable to that of glibenclamide which did not affect antioxidant parameters. Both extracts significantly (p<0.05) raised haematocrit and Total White Blood Cell (TWBC). The MESA produced significant (p<0.05) increases in TWBC while EESA (400 mg kg⁻¹) significantly (p<0.05) reversed the decrease in neutrophil count. Overall, EESA and MESA possess comparable hypoglycaemic and hypolipidaemic effects and show similar potential in attenuating alloxan-induced anaemic state with EESA exhibiting greater antioxidant activity.

Keywords: Sida acuta extracts, diabetes, hyperglycaemia, antioxidant, haematology

INTRODUCTION

Diabetes mellitus, a metabolic disorder characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin

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secretion, insulin action, or both (World Health Organization, 1999) is a progressive disease and is one of the major killers in recent times. World Health Organization (WHO) suggests that worldwide the global population is in the midst of a diabetes epidemic and that the number of cases for diabetes that is currently at 171 million is predicted to reach 366 million by the year 2030 (Wild et al., 2004). Majority (over 90%) of patients with diabetes have type 2 or non-insulin dependent diabetes while the remainder have type 1 or insulin dependent diabetes. Although, the two types of diabetes have distinct pathogenesis, hyperglycaemia and various life-threatening complications resulting from long-term hyperglycaemia are the most common features (Attele et al., 2002). Epidemiological studies (Liu et al., 1993; Klein et al., 1994; Stolk et al., 1995) and clinical trials (Abraira et al., 1995; Ohkubo et al., 1995) strongly support the notion that hyperglycaemia is the principal cause of complications. Effective blood glucose control is the key to preventing or reversing diabetic complications and improving quality of life in patients with diabetes (DCCT Research Group, 1993; De Fronzo, 1999). Thus, sustained reductions in hyperglycaemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications (Gaster and Hirsch, 1998).

Following World Health Organization (1994) recommendations regarding the need to develop and evaluate better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta-cell destruction, promoting beta-cell regeneration or repair and interrupting pathways leading to the various complications of diabetes, several medicinal plants with folkloric use in traditional medicine for the management of diabetes are being investigated in various laboratories worldwide, particularly in developing countries (Sa'idu et al., 2007). Sida acuta is a shrub belonging to Malvaceae family and has been widely used as traditional medicine for treatment of various ailments (Coee and Anderson, 1996; Cacere et al., 1987; Malarajan et al., 2006). Studies have demonstrated some biological activity exhibited by this plant (Benjean et al., 1998; Dassonville et al., 2000; Liggen et al., 2002; Banzozi et al., 2004; Karou et al., 2003) and also revealed the presence of active compounds (Dinan et al., 2001; Jang et al., 2003). In a recent study, Karou et al. (2005) demonstrated the antioxidant activity of Sida acuta, in vitro, using the phosphomolybdenum and ABTS assays. Several studies have demonstrated the antioxidant property of flavonoids and other phenolic compounds both in vivo and in vitro (Messina et al., 1994; Knight and Eden, 1996; Hertog et al., 1993). Similarly, the link between diabetes mellitus and oxidative stress is well established (Cann et al., 2003). Oxidative stress is a major component of molecular and cellular tissue damage mechanisms in a variety of human diseases including diabetes mellitus (Rimm et al., 1996). In the present study, we proposed that the reported in vitro antioxidant activity of Sida acuta may provide some benefit in pathological states associated with free radical production and oxidative stress. We therefore, tested this hypothesis by evaluating the possible protective benefit of this plant and compared the efficacy of the methanolic and ethanolic extracts in experimental diabetes induced by alloxan in Wistar rats.

MATERIALS AND METHODS

Chemicals

Alloxan monohydrate, thiobarbituric acid (TBA) were obtained from Sigma Chemical Co. (USA); reduced glutathione (GSH), Ellman's reagent (5, 5-dithiobis-2-nitrobenzoic acid, DTNB) was purchased from MRS Scientific Ltd (Wickford, UK); trichloroacetic acid (TCA) was obtained from Sigma-Aldrich (Germany); Total Cholesterol (TC), triglyceride (TG),
Uric Acid (UA), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) assay kits were all obtained from Randox Laboratories Ltd (Crumlin, UK). All other chemicals used were of analytical grade.

**Plant Material and Extract Preparation**

*Sidá acuta* was collected from Sagamu environs in Ogun State, South-West Nigeria in year 2008 and study was conducted in the same year. The plant material was authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State and deposited at the herbarium with the voucher number FHI 108210. The leaves were removed from the whole plant and sun-dried. The dry leaves were later pulverized and 100 g was extracted by soaking in 1.2 L of 80% methanol or ethanol at room temperature for 5 days. The solvent-extract mixture was filtered and the extract solution obtained was concentrated at room temperature.

The dry (solid) methanolic and ethanolic extracts were stored at 4°C for subsequent use.

**Phytochemical Screening**

Phytochemical analysis of the leaves of *Sidá acuta* was carried out using simple standard chemical tests following the protocol described by Harborne (1973).

**Animals**

Male and female albino rats of the Wistar strain weighing about 129.7±5.3 were obtained from the colony breed of the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Nigeria. The animals were housed in plastic cages, fed with standard rat chow and drinking water *ad libitum* in an environment with 12 h dark/12 h light cycle during period of acclimatization and throughout the period of experiment.

**Experimental Design**

Diabetes was induced in rats by single intraperitoneal injection of alloxan monohydrate (150 mg kg⁻¹) after an overnight fast. Fasting Blood Glucose Level (BGL) was measured 48 h after injection of alloxan and rats with significant hyperglycaemia (i.e., BGL - 150 mg dL⁻¹) were considered diabetic. The hyperglycaemic (diabetic) rats were then divided into six groups of seven rats per group. The first group of hyperglycaemic rats served as the positive control and received normal saline (10 mL kg⁻¹). Groups II and III were treated with 200 and 400 mg kg⁻¹ body weight of ethanolic extract of *Sidá acuta* (EESA), respectively. Similarly, rats in groups IV and V were treated with 200 and 400 mg kg⁻¹ body weight of methanolic extract of *Sidá acuta* (MESA), respectively. Rats in group VI were treated with glibenclamide (200 mg kg⁻¹). The negative control consists of a separate group (seven rats) of normoglycaemic rats that received normal saline (10 mL kg⁻¹). Extracts and saline administration were given daily for three days by gavage. Fasting BGL of all treatment groups was measured on the 3rd day from blood samples collected by amputation of the tail tip under mild anaesthesia and sterilization with methylated spirit using micro-processor digital blood glucometer (Accu-Chek system, Roche Group, Germany). Animals were subsequently sacrificed by cervical dislocation and blood samples collected by cardiac puncture for haematological and biochemical analysis, respectively.

**Biochemical Evaluation**

Reduced glutathione (GSH) level was assessed by the method of Beutler et al. (1963). Lipid peroxidation was estimated spectrophotometrically by the thiobarbituric acid reactive substance (TBARS) method as described by Varshney and Kale (1990) and expressed in
terms of malondialdehyde (MDA) formed per mg protein. Total Cholesterol (TC) and triglyceride (TG) were estimated following the principle described by Trinder (1969) using commercial kits obtained from Randox Laboratories Ltd, (Crumlin, UK). Uric Acid (UA) was also determined using Randox kit following the principle described by Fossati et al. (1980).

**Estimation of Haematological Parameters**

Packed Cell Volume (PCV), Total White Blood Cell (TWBC) count, Neutrophil Count (NC) and Lymphocyte Count (LC) were carried out according to the methods described by Dacie and Lewis (1991).

**Statistics**

Results were expressed as Mean±Standard error of mean (SEM). Differences between groups were determined by one-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software for windows. Post hoc testing was performed for inter-group comparisons using the Least Significant Difference (LSD) (Levine, 1991) and p-value <0.05 was considered significant.

**RESULTS**

**Phytochemical Analysis**

Phytochemical evaluation of *Sida acuta* leaf revealed the presence of alkaloids, saponins, flavonoids, anthraquinones, cardenolides, polyphenols and tannins.

**Effect of Treatments on Blood Glucose Level**

Table 1 shows the Blood Glucose Levels (BGL) of normal and alloxan-treated rats as well as the effects of EESA and MESA on the hyperglycaemia induced by alloxan. Significant (p<0.001) elevation of BGL by 888.9% was produced following treatment with alloxan (positive control) when compared with normoglycaemic group. The EESA and MESA lowered this alloxan-induced hyperglycaemia by 40.0 and 41.7%, respectively at 200 mg kg⁻¹ dose. The BGL of diabetic rats treated with larger dose (400 mg kg⁻¹) of the extracts remained within the same range as those in the saline-treated diabetic group. Glibenclamide (GBC, 200 mg kg⁻¹) also lowered the BGL of the alloxan-treated rats by 40.3%, an effect comparable with those of EESA and MESA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BGL (mg dl⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Normoglycaemic</td>
<td>57.8±4.2</td>
</tr>
<tr>
<td>Hyperglycaemic</td>
<td></td>
</tr>
<tr>
<td>Alloxan (150 mg kg⁻¹) +</td>
<td></td>
</tr>
<tr>
<td>Saline (10 mL kg⁻¹)</td>
<td>571.6±27.7* (888.9)</td>
</tr>
<tr>
<td>EESA (0.2 g kg⁻¹)</td>
<td>342.7±49.4** (40.6)</td>
</tr>
<tr>
<td>EESA (0.4 g kg⁻¹)</td>
<td>&gt;600 (&gt;5.0)</td>
</tr>
<tr>
<td>MESA (0.2 g kg⁻¹)</td>
<td>333.0±61.0 (41.7)</td>
</tr>
<tr>
<td>MESA (0.4 g kg⁻¹)</td>
<td>541.2±35.9 (5.3)</td>
</tr>
<tr>
<td>GBC (0.2 g kg⁻¹)</td>
<td>341.3±43.5** (40.3)</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (standard error of mean). Expressed as mg dl⁻¹. Values in parenthesis represent percentage increase (+) or decrease (-). *p<0.001 when compared with normoglycaemic (NG) control; **p<0.05 and ***p<0.001 when compared with hyperglycaemic saline-treated (diabetic control) group. BGL: Blood glucose level, GBC: Glibenclamide, EESA: Ethanolic extract of *Sida acuta*, MESA: Methanolic extract of *Sida acuta*.
Lipid Peroxidation, Reduced Glutathione and Uric Acid Levels

The GSH, MDA and UA levels of normoglycaemic and diabetic rats treated with EESA, MESA and GBC are presented in Table 2. The GSH and UA levels significantly (p<0.05) decreased by 33.2 and 27.1%, respectively while MDA level increased significantly (p<0.001) by 189.4% in the saline-treated diabetic rats when compared with the saline-treated normoglycaemic control. The EESA (200 and 400 mg kg\(^{-1}\)) prevented the decrease in GSH by 7.9% and significantly (p<0.01) by 84.8%, respectively in the diabetic rats. This extract (EESA) also significantly (p<0.01) prevented the alloxan-induced decrease in UA by 52.6% at 400 mg kg\(^{-1}\). Also, EESA significantly (p<0.01) prevented lipid peroxidation by lowering MDA level by 58.1% at 200 mg kg\(^{-1}\) in the hyperglycaemic rats. The MESA on the other hand prevented GSH depletion by 33.3 and 23.2% at 200 and 400 mg kg\(^{-1}\) doses, respectively in the alloxan-treated rats, though statistically not significant. In addition, MESA (200 mg kg\(^{-1}\)) non-significantly lowered MDA level by 26.7% and increased UA level by 6.4% in the hyperglycaemic rats. The GBC did not affect GSH and MDA levels of the hyperglycaemic rats but significantly (p<0.01) increased UA level by 57.7% when compared with the saline-treated diabetic rats.

Total Cholesterol and Triglycerides

Table 3 shows results of plasma TC and TG in the various treatment groups. Plasma TC increased significantly (p<0.05) by 55.8% and TG by 122.0% (p<0.001) in the saline-treated

<table>
<thead>
<tr>
<th>Treatments</th>
<th>GSH*</th>
<th>MDA*</th>
<th>UA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycaemic (Saline 10 mL kg(^{-1}))</td>
<td>26.5±3.6</td>
<td>0.06±0.01</td>
<td>10.7±0.5</td>
</tr>
<tr>
<td>Hyperglycaemic:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloxan (150 mg kg(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (10 mL kg(^{-1}))</td>
<td>17.7±1.1*(33.2)</td>
<td>0.19±0.03** (-189.4)</td>
<td>7.8±0.6* (27.1)</td>
</tr>
<tr>
<td>EESA (0.2 g kg(^{-1}))</td>
<td>19.1±3.2 (-7.9)</td>
<td>0.08±0.02*** (58.1)</td>
<td>7.4±1.5 (5.1)</td>
</tr>
<tr>
<td>EESA (0.4 g kg(^{-1}))</td>
<td>32.7±5.7*** (-84.8)</td>
<td>0.20±0.02 (5.2)</td>
<td>11.9±0.8*** (52.6)</td>
</tr>
<tr>
<td>MESA (0.2 g kg(^{-1}))</td>
<td>23.6±0.5 (-33.3)</td>
<td>0.14±0.03 (26.7)</td>
<td>8.3±1.0 (6.4)</td>
</tr>
<tr>
<td>MESA (0.4 g kg(^{-1}))</td>
<td>21.8±2.6 (-23.2)</td>
<td>0.20±0.02 (6.3)</td>
<td>6.3±0.6 (19.2)</td>
</tr>
<tr>
<td>GBC (0.2 g kg(^{-1}))</td>
<td>17.5±3.0 (1.1)</td>
<td>0.18±0.01 (3.1)</td>
<td>12.3±0.4*** (57.7)</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (standard error of mean). *Expressed as μg mL\(^{-1}\), † units mL\(^{-1}\) and ‡ expressed as mg dL\(^{-1}\). Values in parenthesis represent percentage increase (+) or decrease (-). *p<0.05 and **p<0.01 and when compared with normoglycaemic (NG) control; ***p<0.001 when compared with hyperglycaemic saline-treated (diabetic control) group. BGL: Blood glucose level, UA: Uric acid, GBC: Gilbenemide, EESA: Ethanolic extract of S. acuta, MESA: Methanolic extract of S. acuta

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TC*</th>
<th>TG*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycaemic (Saline 10 mL kg(^{-1}))</td>
<td>120.5±11.1</td>
<td>59.0±3.0</td>
</tr>
<tr>
<td>Hyperglycaemic:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloxan (150 mg kg(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (10 mL kg(^{-1}))</td>
<td>187.7±24.2* (-55.8)</td>
<td>131.0±7.0** (-122.0)</td>
</tr>
<tr>
<td>EESA (0.2 g kg(^{-1}))</td>
<td>134.6±17.8 (28.3)</td>
<td>84.8±8.2† (35.9)</td>
</tr>
<tr>
<td>EESA (0.4 g kg(^{-1}))</td>
<td>105.5±32.3*** (43.8)</td>
<td>73.7±6.2†† (43.7)</td>
</tr>
<tr>
<td>MESA (0.2 g kg(^{-1}))</td>
<td>152.0±25.8 (19.9)</td>
<td>73.8±9.3†† (45.6)</td>
</tr>
<tr>
<td>MESA (0.4 g kg(^{-1}))</td>
<td>102.2±16.6† (45.6)</td>
<td>65.8±11.2†† (51.3)</td>
</tr>
<tr>
<td>GBC (0.2 g kg(^{-1}))</td>
<td>120.3±8.8*** (35.9)</td>
<td>73.2±4.8†† (44.1)</td>
</tr>
</tbody>
</table>

Values are Mean±SEM (standard error of mean). *Expressed as mg dL\(^{-1}\). Values in parenthesis represent percentage increase (+) or decrease (-). *p<0.05 and **p<0.01 and when compared with normoglycaemic (NG) control; ***p<0.05, †p<0.01 and ‡p<0.001 when compared with hyperglycaemic saline-treated (diabetic control) group. BGL: Blood glucose level, GBC: Gilbenemide, EESA: Ethanolic extract of S. acuta, MESA: Methanolic extract of S. acuta
hyperglycaemic rats when compared with saline-treated normoglycaemic control. The EBSA lowered the alloxan-induced increase in plasma TC by 28.3 and 43.8% at 200 and 400 mg kg\(^{-1}\) doses, respectively while MESA decreased this parameter by 19.9 and 45.6%, respectively. The decrease in plasma TC produced by these extracts was only significant at 400 mg kg\(^{-1}\). These extracts, however, significantly lowered serum TG [EBSA by 35.9% (p<0.01) and 43.7% (p<0.001) and MESA by 45.6% (p<0.01) and 51.3% (p<0.001)] at both 200 and 400 mg kg\(^{-1}\) doses in the alloxan-treated rats. Also, GBC (200 mg kg\(^{-1}\)) significantly decreased TC and TG by 35.9% (p<0.05) and 44.1% (p<0.001), respectively in the alloxan diabetic rats.

**Blood Parameters**

Alloxan-induced diabetes was associated with decreases in TWBC count and PCV as indicated in Fig. 1. The decrease in PCV was significantly (p<0.001) different for the saline-treated normoglycaemic rats while the reduction in TWBC was not. Both EBSA and MESA significantly (p<0.05 and p<0.001, respectively) prevented the decrease in PCV induced by alloxan both at 200 and 400 mg kg\(^{-1}\). The GBC, which produced effects similar to those of the extracts, significantly (p<0.05) increased the PCV of the alloxan-treated hyperglycaemic rats when compared with the saline-treated diabetic rats. The extracts also prevented the alloxan-induced decrease in TWBC both at 200 and 400 mg kg\(^{-1}\) doses. However, only MESA produced significant (p<0.05) increases in the TWBC when compared with the saline-treated hyperglycaemic rats and the lower dose (200 mg kg\(^{-1}\)) produced

![Fig. 1: Effect of the extract of *Sida acuta* on Packed Cell Volume (PCV) and Total White Blood Cell (TWBC) count in alloxan-induced diabetic rats. Values are Mean±SEM (standard error of mean). *p<0.001 when compared with normoglycaemic (NG) control; **p<0.05 and ***p<0.001 when compared with hyperglycaemic saline-treated (diabetic control) group. BGL: Blood glucose level, GBC: Glibenclamide, EESA: Ethanolic extract of *Sida acuta*, MESA: Methanolic extract of *Sida acuta*](image-url)
Fig. 2. Effect of the extract of *Sida acuta* on neutrophil and lymphphil and lymphocyte count in alloxan-induced diabetic rats. Values are Mean±SEM (standard error of mean).

*p<0.05 when compared with hyperglycaemic saline-treated (diabetic control) group. GBL: Blood glucose level, GBC: Glibenclamide, EESA: Ethanolic extract of *Sida acuta*, MESA: Methanolic extract of *Sida acuta*.

higher effects on both the PCV and TWBC. The GBC also reversed the effect of alloxan on these parameters and significantly (p<0.05) increased PCV when compared with the saline-treated hyperglycaemic rats.

Figure 2 shows the effect of various treatments on neutrophil and lymphocyte counts. Treatment with alloxan decreased neutrophil while increasing lymphocyte count, though values did not change significantly from saline treated normoglycaemic rats. The EESA only significantly (p<0.05) prevented the alloxan-induced decrease in neutrophil count at 400 mg kg⁻¹. Both MESA and GBC did not produce any significant change in both neutrophil and lymphocyte counts in the alloxan-induced hyperglycaemic rats when compared with the saline-treated hyperglycaemic group.

**DISCUSSION**

Earlier studies have reported the presence of biologically active compounds in *Sida acuta* (Dinan et al., 2001; Jang et al., 2003) and in support of these studies, phytochemical analysis of the leaf in our present investigation also revealed the presence of alkaloids, flavonoids, anthraquinones, cardenolides, polyphenols, saponins and tannins. The observed antioxidant property exhibited in vitro by *Sida acuta* in the study by Karou et al. (2005) may be related to the presence of some of these aforementioned compounds as also demonstrated in previously reported studies (Messina et al., 1994; Knight and Eden, 1996). Data from this present study show that both the methanolic and ethanolic leaf extracts of *Sida acuta* are capable of lowering elevated blood glucose level associated with alloxan-induced diabetic state in rats. The ability of these extracts to produce this
(hypoglycaemic) effect appears to be dose related. The hypoglycaemic effects of both MESA and EESA were evident and also similar at the lower dose used in this study. This effect was also comparable to that of GBC. Although, MESA appeared to produce a very mild and negligible reduction in BGL in the alloxan hyperglycaemic rats when compared with the saline-treated diabetic group at 400 mg kg$^{-1}$, it is evident from the result that both extracts were not effective at this dose. The observed hypoglycaemic effect of *Sida acuta* in this study may be related to its alkaloid, flavonoid, tannins or polyphenol content as the hypoglycaemic activity of many plants containing these bioactive compounds have been reported in several studies (Reher et al., 1991; Karawya and Wahab, 1984; Schimizu et al., 1984).

Furthermore, our result is in line with a earlier study that reported the antioxidant activity of *Sida acuta*, *in vivo*, (Karou et al., 2005). This seems to suggest that the observed antihyperglycaemic activity of *Sida acuta* in this study may be due to an antioxidant action. It is known that the cytotoxic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, diacetyl, acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by Fenton reaction. The reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid oxidative stress-induced destruction of β cells with resultant hyperglycaemia (Szkudelski, 2001). This hyperglycaemia further increases the generation of free radicals by glucose auto-oxidation, protein glycation and glycoxidation leading to a decrease in glutathione synthesis and increased lipid peroxidation (Jakus, 2000; Cammi et al., 2003). The EESA attenuated oxidative stress induced by alloxan in this study by significantly reversing GSH depletion in the hyperglycaemic rats. This was also associated with significant lowering of MDA level suggesting a reduction in the extent of membrane lipid peroxidation. The protection against membrane peroxidation by this extract was observed only at the lower (200 mg kg$^{-1}$) dose. Though the reason for this is not clear, it however, supports the observed lack of or poor glycaemic control in the diabetic rats treated with 400 mg kg$^{-1}$ dose of the extract. The EESA seems to exert greater antioxidant effect than MESA in this study. Treatment with MESA also increased GSH level in the alloxan diabetic rats but this, however, was not significant both at 200 and 400 mg kg$^{-1}$ unlike EESA that significantly elevated GSH at 400 mg kg$^{-1}$. Unlike EESA, MESA non-significantly lowered MDA levels at 200 mg kg$^{-1}$ and both produced similar effects at 400 mg kg$^{-1}$. Present result also shows that EESA significantly prevented the decrease in plasma UA level induced by alloxan in the rats unlike MESA which did not produce any significant change in this parameter when compared with the saline-treated diabetic rats. The GBC also prevented the alloxan-induced decrease in plasma UA, an effect similar to that produced by EESA at 400 mg kg$^{-1}$, but unlike EESA, it did not prevent GSH depletion and peroxidation of membrane lipid. The UA has traditionally been considered merely an end product of purine metabolism. However, its function as a biological antioxidant has been increasingly recognized (Cutler, 1984; Buettner, 1993; Fouad, 2005). Urate which is the soluble form of UA has been shown to be capable of scavenging singlet oxygen, superoxide and hydroxyl radicals and can chelate transition metals (Ames et al., 1981; Simie and Jovancovitch, 1989). It is also capable of blocking the reaction by which peroxyxynitrite injures cells (Squadrito et al., 2000). By preventing urate depletion in this study, EESA may further enhance antioxidant defense and reduce oxidant status in the alloxan diabetic rats.

In contrast to the observed difference in the antioxidant potential exhibited by EESA and MESA in this study, both extracts however appear to be effective hypolipidemic agents. The
marked dyslipidemia that characterized alloxan toxicity in this study was significantly ameliorated by EESA and MESA. Plasma TC and TG were dose-dependently deceased by these extracts. It is known that patients with type II diabetes tend to have a characteristic dyslipidemia likely responsible for their being 2 to 4 times more inclined to developing cardiovascular disease than those without the disease (Haffner et al., 1998). In fact, patients with type II diabetes are twice as likely as those without this disease to have elevated TG levels and decreased HDL-C concentrations (Garg and Grundy, 1990). Even when effective glycaemic control is achieved with antidiabetic treatment, dyslipidemia persists in many patients with type II diabetes. It is therefore, important to combine antihyperlipidemic therapy in these patients to reduce long-term cardiovascular risk. The significant hypolipidemic activity of EESA and MESA in the alloxan diabetic rats may confer greater benefit to its overall potential therapeutic value as an antidiabetic agent. The lipid lowering effects of these extracts in this study were comparable with that of GBC.

We observed further in this study that alloxan toxicity produced some haematological alterations in the rats. Although, alloxan toxicity produced a mild increase in lymphocyte count, the overall effect on the haematopoietic system suggests that anaemia and loss of control of inflammatory responses to infections and other forms of cellular injury may accompany alloxan diabetic state. The significant reduction of haematocrit (PCV) in addition to the moderate decreases in TWBC and neutrophil counts following treatment with alloxan provides evidence for this speculation. Both EESA and MESA significantly raised the haematocrit level and may thus prevent alloxan-induced anaemia. The MESA in addition, significantly elevated the TWBC while EESA produced moderate but non-significant increase in this parameter. The effect of EESA was comparable to those of GBC. Only EESA (400 mg kg⁻¹) significantly reversed the decrease in neutrophil count associated with alloxan toxicity in this study. Effects produced on neutrophil count in the hyperglycaemic rats by other treatments were not significant when compared with the saline-treated alloxan diabetic rats.

In conclusion, both the ethanolic and methanolic extracts of Sida acuta exerted protective effects against alloxan-induced diabetes in rats at the lower dose (200 mg kg⁻¹) used in this study. The antioxidant effects demonstrated by these extracts may contribute to this protective effect, although, EESA demonstrated better antioxidant activity than MESA. These extracts also attenuated the dyslipidemia associated with alloxan toxicity thus providing additional beneficial effect in the diabetic rats. The EESA and MESA produced comparable hypoglycaemic and hypolipidaemic effects and showed some potential in attenuating alloxan-induced anaemic state. The MESA, however, showed better potential in reversing the decrease in haematocrit as well as TWBC associated with alloxan toxicity in this study.

REFERENCES


