ALTERATION OF PLASMA BIOCHEMICAL, HAEMATOLOGICAL AND OCULAR OXIDATIVE INDICES OF ALLOXAN INDUCED DIABETIC RATS BY AQUEOUS EXTRACT OF TRIDAX PROCUMBENS LINN (ASTERACEAE)

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ABSTRACT

In this study, the effects of an aqueous extract of the leaves of *Tridax procumbens* on the haematology, plasma biochemistry and ocular indices of oxidative stress was investigated in alloxan induced diabetic rats. Diabetes mellitus was induced by injection of alloxan (80 mg/kg body weight), via the tail vein. The extract was administered orally at 100, 200 and 300 mg/kg (both to normal and diabetic rats), and metformin at 50 mg/kg. On gas chromatographic analysis of the alkaloid fraction of the aqueous extract, thirty nine known alkaloids were detected, consisting mainly of 73.91 % akuamidine, 22.33 % voacangine, 1.27 % echitamine, 0.55 % echitamidine, 0.36 % lupanine, 0.27 % crinamidine, 0.23 % augustamine and 0.10 % 6-hydroxypowelline. Tannic acid and β-sitosterol were detected in high quantities. Compared to Test control, the treatment dose-dependently, significantly lowered (*P*<0.05) plasma glucose, triglyceride, very low density lipoprotein cholesterol, total bilirubin, urea, blood urea nitrogen; plasma alkaline phosphatase, alanine and aspartate transaminases, and ocular superoxide dismutase activities, and lymphocyte count. It also significantly increased (*P*<0.05) plasma calcium and ocular ascorbic acid content, haemoglobin concentration and neutrophil count. This study showed that the extract was hypoglycemic, positively affected the haemopoietic system and integrity and function (dose dependently) of the liver and kidney of the diabetic rats; improved the lipid profile and had no deleterious effect on red cell morphology and protected against oxidative stress in ocular tissues. This study also revealed the presence of pharmacologically active compounds in the leaf extract. All of these, highlight the cardioprotective potential of the leaves of *Tridax procumbens*, and support its use in traditional health care practices for the management of diabetes mellitus.

Keywords: β-sitosterol, hypoglycemia, lipid profile, ocular oxidative stress, tannic acid, *Tridax procumbens* Linn

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by elevated glucose in the plasma resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2004; Centers for Disease Control and Prevention, 2008; Wardlaw, 1999). In Nigeria and other African populations, the past two decades has witnessed the emergence of type 2 diabetes mellitus as a major health problem, affecting about 2-7 % of these populations (Rolfe et al., 1992; Amos et al., 1997). The WHO 2004 report estimated that 1.7 million people in Nigeria had diabetes, with the projection that the number will triple by 2030 (World Health Organization, 2004).
Presently, there is renewed interest in the use of herbal products. This may be attributable to the down turn in the economy, as herbal medicine is perceived to be a cheaper means of treatment (Kamboj, 2000; Acuff et al., 2007). Herbal products can improve glucose metabolism and the overall condition of individuals with diabetes, not only by hypoglycemic effects but also by improving lipid metabolism, antioxidant status, and capillary function (Bailey and Day, 1989). *Tridax procumbens* is one of a number of medicinal herbs that is used in traditional health care practices for the management of diabetes mellitus.

*Tridax procumbens* Linn (Family Asteraceae) is native to Central America and tropical South America, but has spread throughout the tropical and subtropical parts of the world. Its common names are coat buttons, tridax daisy, tridax (United States Department of Agriculture, Agricultural Research Service, 2011). The Ibo people of South Eastern Nigeria call it “mbuli”. It has daisy-like yellow centered white or yellow flowers with three toothed ray floret. The leaves are short, hairy and arrow shaped (Jahangir, 2001). It produces a hard achene fruit that is covered with stiff hairs (Fosberg and Sachet, 1980). It is used as an ornamental or fodder plant, and its leaves are cooked as vegetables (Acharya and Srivastava, 2010; Prajapati et al., 2008). Traditionally, it is used for the treatment of bronchial catarrh, malaria, stomach ache, diarrhoea, epilepsy, diabetes, high blood pressure, haemorrhage, liver problems, and as a hair tonic (Agrawal et al., 2010; Ahirwar et al., 2010; Hemalatha, 2008; Jahangir, 2001; Ravikumar et al., 2005). It possesses antiseptic, insecticidal, parasiticidal properties and has marked depressant action on respiration (Edeoga et al., 2005; Salahdeen et al., 2004; Saxena and Albert, 2005).

Ikewuchi et al. (2009) and Ikewuchi and Ikewuchi (2009a, b) reported the nutrient/nutraceutical potential of the leaves. The protective effects of aqueous extract of the leaves against cholesterol and salt loading in Wistar albino rats (Ikewuchi and Ikewuchi, 2009c: Ikewuchi et al., 2010), as well as its weight reducing (Ikewuchi et al., 2011a) and hypotensive (Ikewuchi et al., 2011b) activities have also been reported. Prabhu et al. (2011) reported the analgesic activity of the leaves. In this study, the effects of an aqueous extract of the leaves of *Tridax procumbens* Linn on haematology, plasma biochemistry and ocular indices of oxidative stress in normal and alloxan-induced diabetic Wistar rats were investigated.

**MATERIALS AND METHODS**

**Preparation of plant extract**

Samples of the fresh *Tridax procumbens* plants (Figure 1) were collected from within the Choba and Abuja Campuses of University of Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, Nigeria, the identity was confirmed/authenticated by Dr. Michael C. Dike of the Taxonomy Unit, Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; and Mr. John Ibe, the Herbarium Manager of the Forestry Department, National Root Crops Research Institute (NRCRI), Umuahia, Nigeria. The samples were rid of dirt and the leaves removed, oven dried at 55°C and ground into powder. The resultant powder was soaked in hot, boiled distilled water for 12 h, after which the resultant mixture was filtered and the filtrate, hereinafter referred to as the aqueous extract was stored in a refrigerator for subsequent use. A known volume of this extract was evaporated to dryness, and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract. The resultant residue was used for the phytochemical study.
Determination of the phytochemical content of the crude aqueous leaf extract

Calibration, identification and quantification

Standard solutions were prepared in methanol for alkaloids and tannins, and methylene chloride for phytosterols. The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards.

Determination of alkaloid composition

The extraction was carried out according to the method of Tram et al. (2002). The alkaloid fraction of the crude aqueous extract was extracted with methanol and subjected to gas chromatographic analysis. Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 (1206) software, to quantify and identify compounds. The column was HP INNOWax Column (30 m × 0.25 mm × 0.25 μm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven was programmed as follows: initial temperature at 60 °C, first ramping at 10 °C/min for 20 min, maintained for 4 min, followed by a second ramping at 15 °C/min for 4 min, maintained for 10 min.

Determination of phytosterol composition

Extraction of oil was carried out according to AOAC method 999.02 (AOAC International, 2002), while the analysis of sterols was carried out according to AOAC method 994.10 (AOAC International, 2000). This involved extraction of the lipid fraction from homogenized sample material, followed by alkaline hydrolysis (saponification), extraction of the non-saponifiables, clean-up of the extract, derivatisation of the sterols, and separation and quantification of the sterol derivatives by gas chromatography (GC) using a capillary column. Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 (1206) software, to quantify and identify compounds. The column was HP INNOWax Column (30 m × 0.25 mm × 0.25 μm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 40 psi. The oven was programmed as follows: initial temperature at 120 °C, followed by ramping at 10 °C/min for 20 min.
**Experimental design for the anti-diabetic study**

Male Wistar albino rats (weighing 180-200 g at the start of the study) were collected from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus. All the experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community Guidelines (EEC Directive of 1986; 86/609/EEC). The rats were weighed and sorted into nine groups (Table 1) of five animals each, so that their average weights were approximately equal. The animals were housed in plastic cages. After a one-week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the animals were fasted overnight, and their baseline fasting blood glucose level (FBS) determined using multiCarein™ triglyceride strips and glucometer (Biochemical Systems International, Arezzo, Italy), by collecting blood via tail cut. Diabetes was induced by injection of a freshly prepared solution of alloxan (80 mg/kg body weight) in normal saline, via the tail vein of five groups, while the other four groups were injected with normal saline alone. The dosage of administration of alloxan was adapted from Radwan (2001). Three days after administration of the alloxan, the animals were again fasted and blood collected via tail cutting blood (Burcelin et al., 1995), for the determination of their fasting glucose levels. It was found that the rats had moderate diabetes, having hyperglycemia (that is, with blood glucose of over 150 % of the control). Then the rats were kept for 3 days to stabilize the diabetic condition (Jyoti et al., 2002) before commencing the treatment, which lasted for ten days. The Diabetmin™ (metformin HCl) and extracts were administered daily by intra-gastric gavages. The dosages of administration of the extracts were adopted and modified from Ikewuchi and Ikewuchi (2009c) and Ikewuchi et al. (2010). The animals were allowed food and water ad libitum. At the end of the treatment period, they were weighed, fasted overnight and anaesthetized by exposure to chloroform. While under anesthesia, they were sacrificed and blood was collected from each rat into heparin and EDTA sample bottles. Whole blood was immediately used to determine the triglyceride levels (using multiCarein strips). Then the eyes were removed and stored for the determination of the ocular markers of oxidative stress. The heparin anti-coagulated blood samples were centrifuged at 1000 g for 10 min, after which their plasma was collected and stored for subsequent analysis, while the EDTA anti-coagulated blood samples were used for the haematological analysis.

**Table 1:** Experimental design for the anti-diabetic screening

| S/N | ID        | Treatment                      |
|-----|-----------|--------------------------------|
| 1   | Normal    | Normal saline and water        |
| 2   | Test control | Alloxan and water            |
| 3   | Reference treatment (Reference) | Alloxan and metformin (50 mg/Kg body weight) |
| 4   | Treatment control I (TPC1) | Normal saline and extract (100 mg/Kg) |
| 5   | Treatment control II (TPC2) | Normal saline and extract (200 mg/Kg) |
| 6   | Treatment control III (TPC3) | Normal saline and extract (300 mg/Kg) |
| 7   | Treatment I (TP1) | Alloxan and extract (100 mg/Kg) |
| 8   | Treatment II (TP2) | Alloxan and extract (200 mg/Kg) |
| 9   | Treatment III (TP3) | Alloxan and extract (300 mg/Kg) |

**Determination of the plasma biochemical indices**

The plasma glucose concentration was determined using the multiCarein™ glucose strips and glucometer (Biochemical Systems International, Arezzo, Italy). Plasma triglyceride concentration was determined using multiCarein™ triglyceride strips and glucometer (Biochemical Systems International, Arezzo, Italy). Plasma total and high density lipoprotein cholesterol concentrations were assayed enzymatically with Randox commercial test kits.
(Randox Laboratories, Crumlin, England, UK). Plasma VLDL- and LDL-cholesterol concentrations were calculated using the Friedewald equation (Friedewald, 1972) as follows:

\[
[\text{LDL cholesterol}] = \frac{[\text{Triglyceride}]}{5.2}
\text{mmol} / L
\]

\[
[\text{Total cholesterol}] - [\text{HDL cholesterol}] - \frac{[\text{Triglyceride}]}{2.2}
\]

(Eqn 1)

\[
[\text{VLDL cholesterol}] = \frac{[\text{Triglyceride}]}{2.2}
\text{mmol} / L
\]

(Eqn 2)

Plasma non-HDL cholesterol concentration was determined as reported by Brunzell et al. (2008):

\[
[\text{Non-HDL cholesterol}] = [\text{Total cholesterol}] - [\text{HDL cholesterol}]
\]

(Eqn 3)

The atherogenic indices were calculated as earlier reported by Ikewuchi and Ikewuchi (2009c, d, 2010) using the following formulae:

Cardiac Risk Ratio (CRR) = \frac{[\text{Total cholesterol}]}{[\text{HDL cholesterol}]}

(Eqn 4)

Atherogenic Coefficient (AC) = \frac{[\text{Total cholesterol}] - [\text{HDL cholesterol}]}{[\text{HDL cholesterol}]}

(Eqn 5)

\text{Atherogenic Index of Plasma (AIP)} = \log(\text{Triglyceride})

\frac{[\text{HDL cholesterol}]}{2.2}

(Eqn 6)

The plasma activities of alanine transaminase, aspartate transaminase, and alkaline phosphatase, were determined using Randox test kits (Randox Laboratories, Crumlin, England, UK). The activities of alanine and aspartate transaminases were respectively measured by monitoring at 546 nm the concentrations of pyruvate and oxaloacetate hydrazones formed with 2,4-dinitrophenylhydrazine. The activity of alkaline phosphatase was determined by monitoring the degradation of p-nitrophenylphosphate to p-nitrophenol, at 405 nm. Plasma total and conjugated bilirubin, urea and total protein concentrations were determined using Randox test kits (Randox Laboratories, Crumlin, England, UK). The wavelength for the determination of conjugated bilirubin and urea was 546 nm and that of total bilirubin was 578 nm. Plasma total protein was determined by the Biuret method using Randox test kits, and the concentration of the resultant coloured complex was measured at 560 nm. Plasma sodium and potassium concentration was determined by flame photometry, according to AOAC Official Method 956.01 (AOAC International, 2006). Plasma calcium concentration was determined by the cresol phthalein complexone method (Baginsky et al., 1973), and the concentration of the resultant complex was measured at 575 nm. Plasma chloride and bicarbonate concentrations were determined by titrimetric methods (Cheesbrough, 2006).

**Determination of the haematological indices**

Haematological indices were determined using Medonic M16 Haematological Analyser (Nelson Biomedical Limited., UK).

**Determination of ocular indices of oxidative stress**

Each eye was homogenized in 4 mL of 0.001 mol/L phosphate buffer (pH 7.4). The resultant homogenate was centrifuged at 1000 x g for 15 min, and the supernatants were collected and stored in the refrigerator for the assays. The protein contents of the homogenates were determined by the biuret method, using Randox test kits (Randox Laboratories, Crumlin, England, UK).

The method adopted for the analysis of malondialdehyde was that of Hunter et al. (1963) as modified by Gutteridge and Wilkins (1982). The concentration of the resultant malondialdehyde - thiobarbituric acid complex (or adduct) was measured at 532 nm. Ascorbic acid content was estimated by iodine titration as reported by Ikewuchi and Ikewuchi (2011). Catalase activity was determined according to the method of Beers and Sizer (1952). The concentration of the residual hydrogen peroxide was measured at 420 nm. Superoxide dismutase
activity was determined according to the method of Misra and Fridovich (1989). The degree of inhibition of the auto-oxidation of adrenaline (which reflects the activity of superoxide dismutase) was determined by measuring the concentration of the resultant adrenochrome, at 520 nm. The amount of enzyme that produced 50 % inhibition was defined as one unit of the enzyme activity.

Statistical analysis of data
All values are reported as the mean ± standard error in the mean (s.e.m.). The values of the variables were analyzed for statistically significant differences using the Student’s \( t \)-test, with the help of SPSS Statistics 17.0 package (SPSS Inc., Chicago III). \( P<0.05 \) was assumed to be significant. Graphs were drawn using Microsoft Office Excel, 2010 software.

RESULTS
Table 2 shows the alkaloid composition of an aqueous extract of the leaves of \( T. \) procumbens. Thirty nine known alkaloids were detected, consisting mainly of 73.91 % akuamidine, 22.33 % voacangine, 1.27 % echitamine, 0.55 % echitamidine, 0.36 % lupanine, 0.27 % crinamidine, 0.23 % augustamine and 0.10 % 6-hydroxypowellite.

Table 2: The composition of the alkaloid fraction of an aqueous extract of the leaves of \( Tridax \) procumbens

| Compounds             | Retention time (min) | Composition (mg/kg) |
|-----------------------|----------------------|---------------------|
| Choline               | 7.096                | 0.007442            |
| Trigonelline          | 7.641                | Not detected        |
| Angustifoline         | 8.109                | Not detected        |
| Sparteine             | 8.952                | 0.002921            |
| Ellipicine            | 9.680                | 0.006273            |
| Lupanine              | 11.041               | 0.105692            |
| 13-\( \alpha \)-Hydrorhombifoline | 11.286        | 0.006579            |
| 9-Octodecanamide     | 12.936               | 0.006590            |
| Dihydro-oxo-demethoxyhæmanthamine | 14.150   | 0.011062            |
| Augustamine           | 14.918               | 0.068184            |
| Oxoassoanine          | 15.395               | 0.009922            |

Table 3 shows the phytosterol and tannin composition of an aqueous extract of the leaves of \( T. \) procumbens. The sterol fraction consisted 100 % of sitosterol, with cholesterol, cholestanol, ergosterol, campesterol, stigmasterol and 5-avenasterol not detected. The tannin fraction consisted 100 % of tannic acid.

Table 3: Composition of the phytosterol and tannin fractions of an aqueous extract of the leaves of \( Tridax \) procumbens

| Compounds        | Retention time (min) | Composition (mg/kg) |
|------------------|----------------------|---------------------|
| Phytosterols     |                      |                     |
| Sitosterol       | 24.790               | 115.79              |
| Tannins          |                      |                     |
| Tannic acid      | 19.201               | 5294.78             |
Table 4 shows the time course of the effect of an aqueous extract of the leaves of *Tridax procumbens* of the plasma glucose levels of normal and alloxan treated rats. On day 0, the plasma fasting glucose concentration of the alloxan treated animals were significantly higher (*P*<0.05) than the untreated animals (Normal, Treatment control 1, Treatment control 2 and Treatment control 3). On days 5 and 10, the plasma fasting glucose levels of the animals administered the extracts were significantly lower (*P*<0.05) than corresponding Test controls and values on day 0. The percentage reductions in plasma fasting glucose levels of the treated rats on days 5 and 10, were significantly higher (*P*<0.05) than the corresponding values of the Test control group.

Table 5 and 6 show the effect of an aqueous extract of the leaves of *Tridax procumbens* on the plasma lipid profiles and atherogenic indices of normal and alloxan treated rats. The 300 mg/kg treatment produced a significantly lower (*P*<0.05) plasma triglyceride and VLDL cholesterol levels, compared to Test control. There were no significant differences in the total-, HDL-, LDL- and non-HDL cholesterol levels, as well as the atherogenic indices of the test groups and Test control. The cardiac risk ratio and atherogenic coefficient of Treatment 1 and the atherogenic index of plasma of Treatment 3 were lower though not significantly than the Test control.

Table 4: Effect of an aqueous extract of the leaves of *Tridax procumbens* on the plasma glucose profiles of normal and alloxan-induced diabetic rats

| Treatment group | Magnitude | Day 0 (mg/dL) | Day 5 | Day 10 |
|-----------------|-----------|--------------|-------|--------|
|                 | Value (mg/dL) | % Reduction | Value (mg/dL) | % Reduction |
| Normal          | 96.00±3.11^a  |             | 98.35±4.63^a |            |
| Test control    | 155.40±48.67^a |           | 108.20±22.7^a |            |
| Reference       | 105.90±0.87^a  |           | 110.00±9.61^a |            |
| Treatment control 1 | 91.75±2.97^a  |           | 106.00±1.76^a |            |
| Treatment control 2 | 85.75±2.29^a  |           | 108.00±3.56^a |            |
| Treatment control 3 | 82.50±1.72^a  |           | 100.00±3.13^a |            |
| Treatment 1     | 146.33±2.14^a  |           | 128.33±2.54 |            |
| Treatment 2     | 138.67±1.97^a  |           | 122.00±3.85 |            |
| Treatment 3     | 233.33±3.42^a  |           | 134.00±5.79 |            |

Values are mean ± s.e.m., *n=5*, per group.

*P*<0.05 compared to corresponding values on day 0.

% reduction = percentage reduction from the corresponding values on day 0.

Table 5: Effect of an aqueous extract of the leaves of *Tridax procumbens* on the plasma lipid profile of normal and alloxan-induced diabetic rats

| Treatment group | Concentration (mmol/L) |
|-----------------|------------------------|
|                 | Triglyceride | Total cholesterol | HDL cholesterol | VLDL cholesterol | LDL cholesterol | Non-HDL cholesterol |
| Normal          | 0.80±0.04^a          | 1.89±0.05^a,b,d | 1.11±0.05^a,b,d | 0.37±0.02^c   | 0.41±0.07^c,k,d | 0.78±0.06^a,c,d,f |
| Test control    | 0.84±0.04^a,b        | 1.74±0.04^a,b,d | 0.73±0.15^a,b,c | 0.38±0.02^a,c | 0.63±0.15^a,b,c,d,f | 1.01±0.14^a,b,c,d,f |
| Reference       | 0.88±0.05^a          | 2.24±0.18^a,b,d | 1.26±0.14^a,b,c,d | 0.40±0.02^a,c | 0.58±0.28^a,b,c,d,f | 0.98±0.26^a,b,c,d,f |
| Treatment control 1 | 0.96±0.05^a,b        | 1.81±0.11^a,b,c,d | 1.16±0.07^a,b,c,d | 0.43±0.02^c   | 0.23±0.05^a,b,c,d | 0.67±0.04^a,b,c,d,f |
| Treatment control 2 | 0.83±0.04^a,b        | 1.86±0.27^a,b,c,d | 0.91±0.05^a,b,c,d | 0.38±0.02^c   | 0.57±0.33^a,b,c,d,f | 0.95±0.32^a,b,c,d,f |
| Treatment control 3 | 0.97±0.07^a          | 1.78±0.03^a,b,c,d | 1.39±0.10^a,b,c,d | 0.44±0.03^a,c,d-f | -0.04±0.11^b | 0.40±0.09^a,b,c,d,f |
| Treatment 1     | 0.82±0.14^a,b,d      | 2.01±0.12^a,b,c | 0.83±0.05^a,b,c,d | 0.37±0.06^a,b,c,d | 0.80±0.10^a,b,c,d | 1.18±0.14^a,b,c,d,f |
| Treatment 2     | 1.02±0.09^a,b        | 2.03±0.05^a,b,c,d | 1.04±0.12^a,b,c,d | 0.46±0.04^a,b,c,d | 0.53±0.19^a,b,c,d,f | 0.99±0.16^a,b,c,d,f |
| Treatment 3     | 0.58±0.01^a          | 1.63±0.06^a,b | 0.91±0.04^a,b,j | 0.27±0.00^b,b,j | 0.45±0.02^j,k,d | 0.72±0.02^a,b,c,d,j |

Values are mean ± s.e.m., *n=5*, per group.

*P*<0.05 compared to corresponding values on day 0.

*P*<0.05 compared to corresponding values on day 0.

% reduction = percentage reduction from the corresponding values on day 0.
Tables 7 and 8 show the effect of an aqueous extract of the leaves of *Tridax procumbens* on the plasma biochemistry of normal and alloxan treated rats. The alkaline phosphatase activity of the animals on Treatment 2 was significantly lower (P<0.05) than Test control, while that of Treatment 3 was significantly higher (P<0.05). The plasma alanine transaminase activity, urea, blood urea nitrogen and total bilirubin levels of Treatment 1 and Treatment 2 were significantly lower (P<0.05) than Test control, while that of Treatment 3 was higher, though not significantly. The aspartate transaminase activity of Treatment 2 and Treatment 3 were significantly lower (P<0.05) than Test control, while that of Treatment 1 was not. The plasma calcium level of the animals on Treatment 1 was significantly higher (P<0.05) than Test control; those of Treatment 2 and Treatment 3 were not significantly different. There were no significant differences between the plasma conjugated and unconjugated bilirubin, unconjugated/conjugated bilirubin ratio, total protein, sodium, potassium and chloride levels of the tests and Test control. The plasma bicarbonate level of the animals on Treatment 3 was significantly lower (P<0.05) than Test control, while those of Treatment 1 and Treatment 2 were not.

Table 6: Effect of an aqueous extract of the leaves of *Tridax procumbens* leaves on the atherogenic indices of normal and alloxan-induced diabetic rats

| Treatment group | Cardiac risk ratio | Atherogenic coefficient | Atherogenic index of plasma proteins |
|-----------------|-------------------|-------------------------|-------------------------------------|
| Normal          | 1.70±0.07bc,cd    | 0.70±0.07abcd           | -0.14±0.02bc,d                     |
| Test control    | 2.74±0.44ab,cd    | 1.74±0.44abcd           | 0.09±0.06bc                         |
| Reference treatment | 1.89±0.32ab,cd    | 0.89±0.32abcd           | -0.15±0.05bc,cd                     |
| Treatment control 1 | 1.59±0.03a,cd    | 0.59±0.03a,b,c         | -0.08±0.04bc,d                     |
| Treatment control 2 | 2.15±0.49ab,cd    | 1.15±0.49abcd           | -0.04±0.03bc,d                     |
| Treatment control 3 | 1.31±0.09b,cd    | 0.31±0.09b             | -0.16±0.02cd                       |
| Treatment 1      | 1.65±0.54ab,cd    | 0.65±0.54abcd           | -0.03±0.07ab,c,d,f                 |
| Treatment 2      | 2.06±0.26ab,cd    | 1.06±0.26abcd           | -0.01±0.04b,c                       |
| Treatment 3      | 1.79±0.01ef      | 0.79±0.01c             | -0.19±0.01                         |

Values are mean ± s.e.m., n=5, per group. Values in the same column with different superscripts are significantly different at P<0.05.

Table 7: Effect of an aqueous extract of the leaves of *Tridax procumbens* on the plasma hepatospecific markers of normal and alloxan-induced diabetic rats

| Treatment group | Alkaline phosphatase activity (U/L) | Alanine transaminase activity (U/L) | Aspartate transaminase activity (U/L) | Total bilirubin (µmol/L) | Direct bilirubin (µmol/L) | Unconjugated bilirubin level (µmol/L) | Free/direct bilirubin ratio | Total protein level (mg/dL) |
|-----------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------|-------------------------|--------------------------------------|-----------------------------|-----------------------------|
| Normal          | 316.2±15.5de                     | 23.5±2.8acd                       | 25.9±0.9bcd                       | 2.9±0.1ab               | 2.3±0.1bc               | 0.7±0.1a                             | 3.4±0.0ab                  | 51±0.8ab                    |
| Test control    | 567.6±121.6de                    | 25.6±6.3c                        | 27.4±0.7ab                        | 3.5±0.2cd               | 2.7±0.2c                | 0.8±0.09ab                          | 3.4±0.0ab                 | 60±2.2ab                    |
| Reference treatment | 176.6±11.2d                    | 21.0±3.0cd                       | 27.1±3.1ab                       | 3.6±0.1cd               | 2.2±0.1c                | 1.4±0.2d                            | 0.7±0.1b                  | 57.6±2.8ab                   |
| Treatment control 1 | 334.0±85.2de                    | 30.5±1.8ad                       | 23.2±1.3bc                       | 2.5±0.1bc               | 1.6±0.2c                | 0.8±0.2ab                           | 0.6±0.2ab                 | 54.7±1.6ab                   |
| Treatment control 2 | 411.9±73.9de                    | 34.9±2.1c                       | 24.9±2.3bc                       | 2.0±0.2b                | 1.3±0.1b               | 0.7±0.2b                            | 0.6±0.2bc                 | 57.8±3.2ab                   |
| Treatment control 3 | 413.1±109.6ab,cd               | 28.5±3.3c                       | 22.2±1.2ac                       | 2.5±0.2ab               | 2.0±0.2c                | 0.6±0.2ab                           | 0.3±0.2abc                | 57.0±0.8c                    |
| Treatment 1      | 575.5±134.9bc,cd               | 23.3±6.2cd                       | 23.9±1.6bc                      | 3.0±0.1ad               | 2.1±0.3b,cd             | 1.0±0.2ab                           | 0.6±0.2abc                | 64.9±1.1c                    |
| Treatment 2      | 391.9±93.4a                    | 22.4±1.1c                       | 17.0±2.3b                        | 3.1±0.2cd               | 2.4±0.1c                | 0.7±0.1ab                           | 0.3±0.1c                  | 52.2±2.5ab                   |
| Treatment 3      | 941.2±107.4e                   | 31.7±3.4d                       | 17.7±1.4c                        | 3.6±0.0c                | 2.5±0.2c                | 1.2±0.1cd                           | 0.5±0.1bc                 | 56.2±1.2c                    |

Values are mean ± s.e.m., n=5, per group. Values in the same column with different superscripts are significantly different at P<0.05.
Table 8: Effect of an aqueous extract of the leaves of *Tridax procumbens* on the plasma electrolyte profiles of normal and alloxan-induced diabetic rats

| Treatment group | Urea (mmol/L) | Blood Urea Nitrogen (BUN, mg/dL) | Calcium (mmol/L) | Sodium (mg/dL) | Potassium (mg/dL) | Chloride (meq/L) | Bicarbonate (meq/L) |
|-----------------|---------------|----------------------------------|-----------------|----------------|-------------------|-----------------|-------------------|
| Normal          | 20.9±2.2c     | 58.7±6.1b                        | 2.1±0.1d        | 129.2±0.8b     | 5.2±0.2a          | 96.8±1.5b       | 18.2±0.6b         |
| Test control    | 43.7±1.3f     | 122.8±3.8g                       | 1.9±0.1e        | 130.0±0.7a     | 5.1±0.1b          | 98.5±0.7e       | 19.5±0.5a         |
| Reference treatment control 1 | 12.7±2.3d  | 35.6±6.4c                        | 2.0±0.1b        | 148.7±12.4ab   | 9.2±1.9b          | 114.7±10.1ab    | 18.0±0.5c         |
| Treatment control 1 | 14.7±0.7d    | 41.4±2.0c                        | 2.1±0.1e        | 142.0±8.5ab    | 5.2±0.4ab         | 146.5±37.3ab    | 18.0±2.2ab        |
| Treatment control 2 | 15.7±0.9a,b,c| 44.0±2.6a                        | 2.1±0.0a        | 130.8±1.2ab    | 5.0±0.2ab         | 102.8±1.4ab     | 15.3±1.6abc       |
| Treatment control 3 | 17.1±1.0b     | 48.0±2.9a                        | 1.9±0.1f        | 128.0±1.8ab    | 5.7±0.5ab         | 97.3±1.6ab      | 14.3±1.0         |
| Treatment 1     | 26.6±3.9a,b,c,h | 74.7±11.1a                        | 2.2±0.0a        | 133.5±1.1a     | 5.3±0.2ab         | 97.7±1.3ab      | 17.3±0.7abc       |
| Treatment 2     | 23.8±1.8a,n   | 66.7±4.9a                        | 2.1±0.4b        | 131.3±1.0ab    | 4.8±0.4b          | 102.8±1.7c      | 17.3±0.9abc       |
| Treatment 3     | 54.2±0.05k   | 152.1±1.4b                       | 1.9±0.0k        | 128.0±0.7b     | 5.4±0.1a          | 97.0±2.8ab      | 16.0±0.5df        |

Values are mean ± s.e.m., n=5, per group.

Table 9 shows the effect of an aqueous extract of the ocular markers of oxidative stress of normal and alloxan treated rats. The ocular ascorbic acid contents of the test animals were higher than the Test control; with Treatment 2 being significantly so \( P<0.05 \). There were no significant differences in the ocular malondialdehyde levels of all the groups. The ocular catalase activities of the test animals were lower though not significantly than the Test control. The ocular superoxide dismutase activities of the test animals were lower than the Test control; with Treatment 3 being significantly so \( P<0.05 \).

Table 10 shows the effect of an aqueous extract of the ocular markers of oxidative stress of normal and alloxan treated rats. The haematocrit, red cell and monocyte counts of Treatment 2 and Treatment 3 were higher though not significantly, than Test control, while that of Treatment 1 was lower. The haemoglobin concentrations of Treatment 2 and Treatment 3 were significantly higher \( P<0.05 \) than Test control, while that of Treatment 1 was lower. There were no significant differences in the total white cell counts, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentrations of the test groups and Test control. The neutrophil count of Treatment 2 was significantly higher \( P<0.05 \) than Test control, while those of Treatment 1 and Treatment 3 were not. The lymphocyte count of Treatment 2 was significantly lower \( P<0.05 \) than Test control, while those of Treatment 1 and Treatment 3 were not. The platelet counts of the test groups were higher (though not significantly) than the Test control. Figure 1 shows the frequency distribution of the effect of an aqueous extract of the leaves of *Tridax procumbens* on the cell morphology of normal and alloxan treated rats. All the cells from the animals on Treatment 2 and Treatment 3 were normochromic and normocytic, while those from Treatment 1 were 66 % hypochromic and 33 % normochromic; and 25 % poikilocytic, 50 % normocytic and 25 % anisocytic. This result shows that the extract had no deleterious effect on red cell morphology.
Figure 1: Frequency distribution of the effect of an aqueous extract of the leaves of Tridax procumbens on red cell morphology of normal and alloxan-induced diabetic rats. TPC1 = Treatment control 1, TPC2 = Treatment control 2, TPC3 = Treatment control 3, TP1 = Treatment 1, TP2 = Treatment 2, TP3 = Treatment 3.

Table 9: Effect of an aqueous extract of the leaves of Tridax procumbens on ocular markers of oxidative stress in normal and alloxan-induced diabetic rats

| Treatment group         | Magnitude                  |
|-------------------------|----------------------------|
|                         | Ascorbic acid content      | Malondialdehyde content | Catalase activity       | SOD activity         |
|                         | (µmole/g protein)          | (µmol/g protein)         | (Units/mg protein)      | (Units/mg protein)   |
| Normal                  | 11.23±1.71^{a,b,c,h}       | 0.09±0.01^{a,b,c,d}      | 20.60±3.61^{a,b,c}      | 0.20±0.02^{a,b,c}    |
| Test control            | 8.72±0.34^{a,f}            | 0.09±0.01^{c,d}          | 25.84±3.68^{a,b,c,d}    | 0.16±0.02^{c,d,i,k}  |
| Reference treatment     | 8.30±0.42^{c,f,k}          | 0.07±0.01^{d}            | 23.43±0.49^{c,d}        | 0.18±0.00^{e}       |
| Treatment control 1     | 11.39±3.00^{a,b,c,h}       | 0.12±0.01^{a,b,c}        | 30.00±1.57^{b}          | 0.23±0.014^{b,c}    |
| Treatment control 2     | 12.31±1.43^{a,b}           | 0.08±0.02^{c,d}          | 22.62±2.70^{a,b,c,d}    | 0.15±0.03^{b,d,f,h,k}|
| Treatment control 3     | 7.50±0.05^{h,k}            | 0.15±0.02^{b}            | 19.39±0.17^{a}          | 0.07±0.00^{h}       |
| Treatment 1             | 10.32±0.92^{a,b,c}         | 0.11±0.01^{a,c}          | 23.75±1.53^{c,d}        | 0.14±0.01^{h,k}     |
| Treatment 2             | 19.43±3.33^{b}            | 0.09±0.01^{a,b,c,d}      | 18.5±1.29^{a}           | 0.12±0.01^{d}       |
| Treatment 3             | 12.90±2.48^{a,b,c,h}       | 0.12±0.01^{a}            | 19.48±0.12^{a}          | 0.05±0.01^{l}       |

Values are mean ± s.e.m., n=5, per group.

\(^{a,b,c}\)Values in the same column with different superscripts are significantly different at \(P<0.05\).

**DISCUSSION**

Alloxan induced diabetes mellitus is often characterized by decreased levels of insulin and high density lipoprotein, hyperglycemia, elevated triglycerides and total cholesterol (Hemalatha, 2008). The high percentage reduction in plasma glucose levels, produced by the extract in this study, supports the use of the leaves in the management of diabetes mellitus. The extract may exert its antihyperglycemic activity by stimulating insulin secretion from pancreatic \(\beta\) cells exerting insulin like activity, or by converting pro-insulin to insulin, or alternatively, by inhibiting hepatic gluconeogenesis. The hypoglycemic effect of the extract may have been produced by the saponins (Ikewuchi et al., 2009), tannic acid and \(\beta\)-sitosterol (Table 3) present in the leaves. Saponins (Soetan, 2008), tannic acid (Liu et al., 2001, 2005; Muthusamy et al., 2008; Pereira et al., 2009) and \(\beta\)-sitosterol (Ivorra et al., 1988; Beta-sitosterol Monograph, 2001) are compounds with established hypoglycemic activity. The extract may exert its antihyperglycemic activity by enhancing glucose uptake (by tannic acid), stimulation
of insulin secretion from pancreatic β cells (by β-sitosterol) and insulin like activity, or by conversion of pro-insulin to insulin, or alternatively, by inhibition of hepatic gluconeogenesis.

A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases (Dobiásová, 2004; McBride, 2007; Martirosyan et al., 2007); and is often associated with hypertension (Lopes et al., 1997; Zicha et al., 1999), abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (McBride, 2007; Franz et al., 2002; Shen, 2007). The 300 mg/kg treatment significantly reduced plasma levels of triglycerides (Table 4). This effect may have been mediated by the flavonoid (Ikewuchi et al., 2009) and tannic acid (Table 3) contents of the leaves. Flavonoids (Middleton et al., 2000) and tannic acid (Park et al., 2002) are reported to decrease plasma levels of triglycerides.

High plasma levels of VLDL cholesterol is a risk factor for cardiovascular disease (Ademuyiwa et al., 2005; Lichtenstein et al., 2006) and often accompanies diabetes mellitus (Brunzell et al., 2008; Shen, 2007; Rang et al., 2005) and obesity (Krauss et al., 2006). In this study, we observed a significantly lower plasma VLDL cholesterol level in the treated animals. This cholesterol lowering effect of the extract may be due to its content of β-sitosterol and tannic acid (see Table 3) which are known to have cholesterol lowering and atheroprotective activity (Dillard and German, 2000; Yugarani et al., 1993; Piironen et al., 2000; Park et al., 2002; Bouic, 2003; Berger et al., 2004; Basu et al., 2007). Thus, anyone or a combination of some or all of the above mentioned components could have been responsible for the hypocholesterolemic effect of the extract, observed in this study.

The treatment dose dependently lowered (though not significantly) the atherogenic indices of the animals. Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Dobiáslová, 2004; Martirosyan et al., 2007; Brehm et al., 2004; Usoro et al., 2006). Low atherogenic indices are protective against coronary heart disease (Usoro et al., 2006).

These results indicate that the extract has a dose dependent positive effect on the integrity and function of the liver and kidney of the diabetic rats. The extract improved the lowered plasma calcium level produced by the diabetic condition. The significance of this cannot be overemphasized. Many neuromuscular and other cellular functions depend on the maintenance of the ionized calcium concentration in the extracellular fluid (Crook, 2006). Calcium fluxes are also important mediators of hormonal effects on target organs through several intracellular signaling pathways, such as the phosphoinositide and cyclic adenosine monophosphate systems (Crook, 2006; FAO, 2004). The extract may have achieved this by affecting parathyroid hormone secretion. This hormone increases the renal tubular reabsorption of calcium, promotes intestinal calcium absorption by stimulating the renal production of 1,25-dihydroxyvitamin D or calcitriol (1,25-(OH)2D), and, if necessary, resorbs bone (Crook, 2006; Brown and Hebert, 1997). Collectively, these results indicate that the extract dose dependently improved the liver and kidney functions.

Ascorbic acid functions as an important component of the cellular defense against oxygen toxicity and lipid peroxidation which is caused by the free radical mechanism. Reduced levels and the altered metabolic turnover of ascorbic acid have been reported in diabetic patients. A decrease in the plasma concentration of ascorbic acid has been observed in diabetic patients (Atalay and Laaksonen, 2002; Samuel et al., 2010). The uptake of ascorbic acid into the cell is mediated by processes which are re-
Table 10: Effect of an aqueous extract of the leaves of *Tridax procumbens* leaves on the haematological indices of normal and alloxan-induced diabetic rats

| Parameter                                      | Normal                | Test control          | Reference             | Treatment control 1 | Treatment control 2 | Treatment control 3 | Treatment 1   | Treatment 2   | Treatment 3   |
|------------------------------------------------|-----------------------|-----------------------|-----------------------|---------------------|---------------------|---------------------|----------------|----------------|----------------|
| Haematocrit (%)                                | 34.4±2.6abc           | 38.8±2.2abc           | 31.0±0.5a             | 39.5±2.4abc         | 36.5±2.6abc         | 39.0±1.6abc         | 35.0±2.5abc   | 42.3±1.3a     | 42.0±1.0a     |
| Haemoglobin concentration (g/dL)               | 12.0±0.5abc           | 12.3±0.5ac            | 11.4±0.1c             | 13.0±0.5abc         | 12.2±0.6abc         | 12.3±0.4abc         | 11.6±0.7c     | 13.9±0.3b     | 13.4±0.3b     |
| Red cell count (x 10⁹ cells/L)                 | 5.7±0.2abc            | 6.7±0.4abc            | 5.5±0.0d              | 7.0±0.5f            | 6.4±0.4abcdef       | 6.9±0.2f            | 6.3±0.4cdef   | 7.4±0.2b     | 7.2±0.4b     |
| Total white cell count (x10⁹ cells/L)          | 9.7±1.1a              | 11.9±1.3b             | 10.2±1.0ab            | 18.7±3.1b           | 17.3±3.6abc         | 13.7±1.3b           | 12.0±1.4ab    | 11.6±3.9ab    | 10.6±0.3b     |
| Neutrophils (%)                                | 5.3±1.0abc            | 3.7±0.4c              | 5.5±1.4c              | 5.0±1.9abc          | 6.4±1.5abc          | 11.3±1.3a           | 5.3±1.0abc    | 10.3±0.2b     | 4.5±0.2b     |
| Lymphocytes (%)                                | 81.7±3.7abc           | 83.3±2.3a             | 77.5±1.4abc           | 84.0±4.1abc         | 79.4±2.5abc         | 72.7±0.8b           | 85.3±2.9abc   | 75.3±3.3bc    | 81.0±1.6abc   |
| Monocytes (%)                                  | 12.8±2.7abc           | 12.8±2.0abc           | 15.6±1.4a             | 11.0±2.5abc         | 14.9±1.5abc         | 16.6±0.7b           | 9.5±1.8b     | 14.5±3.3abc   | 14.5±1.5abc   |
| Eosinophils (%)                                | 0.0±0.0a              | 0.0±0.0a              | 0.0±0.0a              | 0.0±0.0a            | 0.0±0.0a            | 0.0±0.0a            | 0.0±0.0a     | 0.0±0.0a     | 0.0±0.0a     |
| Basophils (%)                                  | 0.0±0.0a              | 0.0±0.0a              | 0.0±0.0a              | 0.0±0.0a            | 0.0±0.0a            | 0.0±0.0a            | 0.0±0.0a     | 0.0±0.0a     | 0.0±0.0a     |
| Mean cell volume (fL)                          | 58.7±3.2abc           | 57.5±1.5abc           | 56.5±0.5a             | 57.5±1.9abc         | 57.0±1.0abc         | 59.9±1.6b           | 55.5±1.1abc   | 57.5±1.3abc   | 57.8±1.2abc   |
| Mean cell haemoglobin concentration (g/dL)     | 35.5±1.2abc           | 34.1±0.5abc           | 36.3±0.6a             | 33.4±0.4abc         | 32.2±1.7abc         | 34.5±1.0abc         | 36.5±0.7abc   | 34.6±0.3d     | 33.9±0.3f     |
| Mean cell haemoglobin (pg/cell)                | 20.8±0.7abc           | 19.8±0.3abc           | 20.5±0.2a             | 19.1±0.5b           | 19.7±0.1b           | 19.4±0.1b           | 20.1±0.6abc   | 19.9±0.4abc   | 19.5±0.4b     |
| Platelet count (x10³ cells/mm³)                | 447.8±33.3abc         | 340.8±54.1cde         | 343.0±8.9d            | 494.0±29.5bc        | 425.5±54.8abcde     | 581.9±24.4b         | 396.0±26.5abc | 503.7±66.3abc | 431.5±4.9abc |

Values are mean ± s.e.m., n=5, per group. a,b,cValues in the same row with different superscripts are significantly different at P<0.05.
lated to glucose transport and it has been shown that the high extracellular glucose concentration in diabetes may further impair the cellular uptake of ascorbic acid and accentuate the problems which are associated with its deficiency (Samuel et al., 2010). Studies show that ascorbic acid protects the lens and other tissues of the eye from light damage (Varma, 1991; Taylor, 1993). So, these increases caused by the extract, portends a consolidation of antioxidant status of the tissues, hence protection of these tissues from free radical damage. This high ocular content of ascorbic acid may have been produced by the high content of ascorbic acid in the leaves (Ikewuchi and Ikewuchi, 2009a).

Numerous reports indicate variations in the levels of antioxidants in the diabetic patients (Samuel et al., 2010; Hartnett et al., 2000). Therefore, though the lowered enzyme activities produced by the extract may portend compromised antioxidant protection; the antioxidant vitamins in the test rats clearly indicate that the extract protects against oxidative stress in ocular tissues and probably may have cushioned against the compromised antioxidant enzymes.

The extract had a dose dependent positive effect on the haemopoietic system of the test rats. It increased (though not significantly) the red cell mass, haematocrit and monocyte counts, and platelet number; significantly increased haemoglobin concentration and neutrophil count, while decreasing lymphocyte count. That the extract improved the haemoglobin concentration and haematocrit highlights the potential of the plant in the management of anaemia. This property may be attributable to the iron contained by the leaves (Ikewuchi and Ikewuchi, 2009b).

**CONCLUSIONS**

This study showed that the extract was hypoglycemic, positively affected the haemopoietic system and integrity and function (dose dependently) of the liver and kidney of the diabetic rats; improved the lipid profile and had no deleterious effect on red cell morphology. The profiles of malondialdehyde and antioxidant vitamins in the test rats clearly indicate that the extract protects against oxidative stress in ocular tissues. This study also revealed the presence of pharmacologically active compounds in the leaf extract. All of these, highlight the cardioprotective potential of the leaves of *Tridax procumbens*, and support its use in traditional health care practices for the management of diabetes mellitus.

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