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Positive moderation of the hematology, plasma biochemistry and ocular indices of oxidative stress in alloxan-induced diabetic rats, by an aqueous extract of the leaves of *Sansevieria liberica* Gerome and Labroy

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ABSTRACT

Objective: To investigate the ability of an aqueous extract of the leaves of *Sansevieria liberica* (*S. liberica*) to alter the hematology, plasma biochemistry and ocular indices of oxidative stress in alloxan-induced diabetic rats. **Method:** 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 body weight (both to normal and diabetic rats), and metformin at 50 mg/kg body weight. **Results:** Compared to test control, the treatment dose dependently, significantly lowered ($P<0.05$) ocular malondialdehyde content, atherogenic indices, red cell, total white cell and lymphocyte counts, mean cell hemoglobin concentration; and plasma levels of glucose, triglyceride, total-, very low density lipoprotein-, low density lipoprotein- and non-high density lipoprotein cholesterols, total, conjugated and unconjugated bilirubin, sodium, urea, blood urea nitrogen, as well as plasma activities of alkaline phosphatase, alanine and aspartate transaminases. However, the treatment significantly increased ($P<0.05$) hematocrit, hemoglobin concentration, mean cell hemoglobin, and mean cell volume, neutrophil and monocyte counts, and plasma levels of high density lipoprotein cholesterol, potassium, chloride, calcium, bicarbonate and total protein, ocular ascorbic acid content and ocular activities of catalase and superoxide dismutase. This study showed the hypoglycemic, hypolipidemic, immune-modulating, ocular-, hepato-renal and cardio-protective potentials of the extract. **Conclusions:** All these, support the use of the leaves of *S. liberica* in African traditional health care practices for the management of diabetes mellitus.

1. Introduction

Sansevieria liberica (*S. liberica*) (family Agavaceae, Ruscaceae or Dracaenaceae), is one of the bowstring hemp species[1], with concave, short petioled leaves that are in part transversely banded with light and dark green, also linearly striated with whitish to light green and dark green striations[2]. This plant has long rhizomes with long fibrous roots and a rapid rate of growth. The fruit is a red or orange berry. It is a rather stout herb with several stiff red-margined leaves about 2 feet high arising from the creeping plant, and 50-80 cm long inflorescence (longer than the leaves) with abundant white flowers. They are grown as ornamental plants[3], and are widely distributed throughout the tropics. Their leaves are very rich in fibers[4,5], protein[5], potassium, calcium, magnesium, vitamin C, biotin, and riboflavin[6]. The leaves also contain alkaloids, allicins, carotenoids, flavonoids, glycosides, saponins and tannins[5,7]. Fibers for local use are obtained from the leaves in various countries for making string, nets, coarse fabrics and bows. Osabohien[8] reported that bowstring hemp fiber produced appreciable reinforcement of natural rubber, though inferior to CB(N330) filler, but gave harder vulcanizates. In traditional health care practice, the leaves are used as pain killers, and in the treatment of small pox, chicken pox, measles and most venereal diseases. The pressed juice of the leaves is dropped in the eyes and ears for the treatment of infections and inflammations. The fumes from the burning leaves are inhaled to relieve feverish headaches and cold. A decoction of the roots is drunk as a remedy for convulsions and as a vermifuge. In Nigeria,

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the leaves and roots of *S. liberica* are used in traditional health care practice for the treatment of asthma, abdominal pains, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, diabetes mellitus, monorrhagia, piles, sexual weakness, wounds of the foot, and alleviating the effects of snake bites[4,8-11]. The hypotensive effect of aqueous extract of the leaves[12], as well as their ability to moderate hematological indices and plasma chemistry in salt-loaded rats[13], has been reported. The present study investigated the effects of an aqueous extract of the leaves of *S. liberica* on hematology, plasma biochemistry and ocular indices of oxidative stress in alloxan-induced diabetic Wistar rats

2. Materials and methods

2.1. Chemicals

All chemicals used were of analytical grade.

2.2. Preparation of plant extract

Samples of fresh *S. liberica* plants (Figure 1) were procured from a horticultural garden by Air Force Gate, Aba Road, Port Harcourt, and another at the University of Port Harcourt's Abuja campus, University of Port Harcourt, Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, the identity was confirmed/authenticated by Dr. Michael C. Dike of 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. They were rid of dirt and the leaves were removed, oven dried at 55 ℃ 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 the 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.

Figure 1. *S. liberica*.

2.3. Experimental design

Wistar albino rats (weighing 180-205 g at the start of the study) were obtained from the animal house of the Department of Physiology, University of Nigeria, Enugu Campus. Studies were conducted in compliance with the applicable laws and regulations for handling experimental animals. 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 at the animal house of the Department of Biochemistry, University of Port Harcourt. 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 determined using a glucometer, 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 six groups, while the control rats were injected with normal saline alone. The dosage of administration of alloxan was adopted from Radwan[14]. Three days after administration of the alloxan, the animals were again fasted and blood collected via tail cutting blood[15], 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 180% of the control). Then the rats were kept for

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 (LLC1)	Normal saline and extract (100 mg/kg)
5	Treatment control II (LLC2)	Normal saline and extract (200 mg/kg)
6	Treatment control III (LLC3)	Normal saline and extract (300 mg/kg)
7	Treatment I (LL1)	Alloxan and extract (100 mg/kg)
8	Treatment II (LL2)	Alloxan and extract (200 mg/kg)
9	Treatment III (LL3)	Alloxan and extract (300 mg/kg)

3 d to stabilize the diabetic condition[16] before commencing the treatment, which lasted for ten days. The DiabetminTM (metformin HCl) and extracts were administered daily by intra-gastric gavages. The dosages of administration of the extracts were adopted and modified from Ikewuchi *et al*[12,13]. The animals were allowed food and water ad libitum. The fasting glucose levels were taken on days 5 and 10. The animals were allowed normal feed and water ad libitum. At the end of the treatment period, the rats were weighed, fasted overnight and anaesthetized by exposure to chloroform. While under anesthesia, they were painlessly 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 1 000 *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 hematological analysis.

2.4. Determination of the plasma biochemical indices

The plasma glucose concentration was determined using the multiCareinTM glucose strips and glucometer (Biochemical Systems International, Arezzo, Italy). Plasma triglyceride concentration was determined using multiCareinTM triglyceride strips and glucometer. Plasma total and high density lipoprotein cholesterol concentrations were assayed enzymatically with Randox commercial test kits (Randox Laboratories Ltd., Crumlin, England, UK). In the presence of magnesium ions, low density lipoproteins (LDL and VLDL) and chylomicrons fractions were precipitated quantitatively by the addition of phosphotungstic acid. After centrifugation, the cholesterol concentration of the high density lipoprotein (HDL) fraction, which remained in the supernatant, was determined, as in total cholesterol. The cholesterol released by enzymatic hydrolysis was oxidized with the concomitant release of hydrogen peroxide, whose breakdown led to the conversion of 4-aminoantipyridine to quinoneimine (the indicator) whose concentration was determined spectrophotometrically at 500 nm.

Plasma VLDL- and LDL-cholesterol concentrations were calculated using the Friedewald equation[17] as follows:

$$[\text{LDL cholesterol}]\ (\text{mmol/L}) = [\text{Total cholesterol}] - [\text{HDL cholesterol}] - \frac{[\text{Triglyceride}]}{2.2} \quad \text{[Eqn 1]}$$

$$[\text{LDL cholesterol}]\ (\text{mmol/L}) = \frac{[\text{Triglyceride}]}{2.2} \quad \text{[Eqn 2]}$$

While the plasma non-HDL cholesterol concentration was determined as reported by Brunzell *et al*[18]:

$$[\text{Non-HDL cholesterol}] = [\text{Total cholesterol}] - [\text{HDL cholesterol}] \quad \text{[Eqn 3]}$$

The atherogenic indices were calculated as earlier reported by Ikewuchi and Ikewuchi[19,20,21] using the following formulae:

$$\text{Cardiac Risk Ratio (CRR)} = \frac{[\text{Total Cholesterol}]}{[\text{HDL cholesterol}]} \quad \text{[Eqn 4]}$$

$$\text{Atherogenic Coefficient (AC)} = \frac{[\text{Total cholesterol}] - [\text{HDL cholesterol}]}{[\text{HDL cholesterol}]} \quad \text{[Eqn 5]}$$

$$\text{Atherogenic Index of Plasma (AIP)} = \log \frac{[\text{Triglyceride}]}{[\text{HDL cholesterol}]} \quad \text{[Eqn 6]}$$

The plasma activities of alanine and aspartate transaminases, and alkaline phosphatase, were determined using Randox test kits (Randox Laboratories Ltd., 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 creatinine concentrations were determined using Randox test kits (Randox Laboratories Ltd., 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

Table 2.
Effects of an aqueous extract of the leaves of *S. liberica* leaves on the plasma glucose profiles of normal and alloxan treated rats.

Treatment group	Day 0 (mg/dL)	Day 5		Day 10	
		Value (mg/dL)	Reduction (%)	Value (mg/dL)	Reduction (%)
Normal	96.00±2.92^{a}	98.35±4.46^{a,d}	−2.61±7.29^{a}	104.20±5.07^{a}	−8.73±8.17^{a}
Test control	155.40±5.73^{c,e}	208.00±5.10^{c,*}	−33.94±4.13^{c}	179.50±8.20^{c,*}	−15.67±7.36^{a}
Reference	125.90±1.95^{d}	89.33±2.10^{d,e,*}	29.05±0.64^{d}	110.00±7.52^{a,d,*}	12.56±6.88^{c}
LLC1	83.20±9.86^{b}	89.40±8.62^{b,d,e}	−7.99±10.19^{a,e}	91.80±6.98^{e,*}	−10.84±6.91^{a}
LLC2	78.75±5.31^{b}	94.00±7.65^{a,d,e,*}	−19.62±10.03^{e}	94.40±5.68^{b,e,*}	−20.18±8.92^{a}
LLC3	99.00±4.00^{a}	89.60±9.04^{a,d,e}	9.41±9.25^{e,f}	102.80±8.87^{a,b}	−3.99±9.92^{a}
LL1	137.40±9.53^{d}	109.33±8.64^{b,f,*}	20.08±9.11^{d,f}	112.00±3.46^{d,*}	18.16±6.38^{c}
LL2	153.33±7.85^{e}	88.67±7.12^{e,*}	41.89±7.42^{b}	99.00±4.90^{a,b,e,*}	35.36±3.15^{b}
LL3	144.20±9.31^{c}	104.33±7.74^{a,f,*}	27.58±4.27^{d}	119.80±6.34^{d,*}	16.58±7.68^{c}

Values are mean±s.d., n=5, per group. ^{a,b}Values in same column with different superscripts letters are significantly different at P<0.05. *P<0.05 compared to corresponding values on day 0. Reduction (%) = percentage reduction from the corresponding values on day 0.

using Randox test kits, and the concentration of the resultant colored complex was measured at 560 nm.

Plasma sodium and potassium concentration was determined by flame photometry, according to AOAC Official Method 956.01[22]. Plasma calcium concentration was determined by the cresol phthalein complexone method[23], and the concentration of the resultant complex was measured at 575 nm. Plasma chloride and bicarbonate concentrations were determined by titrimetric methods[24].

2.5. Determination of the hematological indices

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

2.6. Determination of ocular indices of oxidative stress

Each eye was homogenized in 4 mL of 1 mmol/L phosphate buffer (pH 7.4). The resultant homogenate was centrifuged at 1 000 *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.

The method adopted for the analysis of malondialdehyde was that of Hunter *et al*[25] as modified by Gutteridge and Wilkins[26]. 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[27], and Ikewuchi *et al*[28]. Catalase activity was determined according to the method of Beers and Sizer[29]. 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[30]. 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.

2.7. Statistical analysis of data

All values are reported as the mean±s.d. (standard deviation). The values of the variables were analysed for statistically significant differences using the Student's *t*-test, with the help of SPSS Statistics 17.0 package (SPSS Inc., Chicago Ill). $P<0.05$ was assumed to be significant.

3. Results

The time course of the hypoglycemic effect of an aqueous extract of the leaves of *S. liberica* on normal and alloxan-induced diabetic rats is presented in Table 2. On day 0, the plasma fasting glucose concentration of the alloxan treated animals were significantly higher ($P<0.05$) than the untreated animals (normal, LLC1, LLC2 and LLC3). On days

Table 3.
Effects of an aqueous extract of the leaves of *S. liberica* on the plasma lipid profile of normal and alloxan treated rats.

Treatment group	Concentration (mmol/L)					
	Triglyceride	Total cholesterol	HDL cholesterol	VLDL cholesterol	LDL cholesterol	Non-HDL cholesterol
Normal	0.84±0.09^{a}	1.74±0.08^{a}	1.11±0.04^{a}	0.38±0.04^{a}	0.25±0.10^{a,d}	0.63±0.11^{a,d}
Test control	1.04±0.06^{c}	2.24±0.14^{c}	0.71±0.05^{c}	0.48±0.03^{c}	1.06±0.12^{c}	1.53±0.10^{c}
Reference	0.88±0.03^{a}	1.89±0.12^{a,b}	1.24±0.07^{c}	0.40±0.02^{a}	0.25±0.08^{d}	0.65±0.07^{d}
LLC1	1.00±0.15^{a,c}	1.90±0.16^{a,b}	1.09±0.15^{a,c}	0.45±0.07^{a,c}	0.36±0.02^{a,e}	0.81±0.06^{a}
LLC2	0.87±0.08^{a}	1.89±0.07^{b}	1.21±0.03^{c}	0.40±0.04^{a}	0.28±0.10^{a,d,f}	0.68±0.09^{a,d}
LLC3	1.00±0.14^{a,c}	1.92±0.13^{b}	1.19±0.07^{a,c}	0.46±0.07^{a,c}	0.28±0.05^{d}	0.73±0.07^{a,d}
LL1	1.04±0.09^{c}	1.82±0.05^{b}	0.87±0.05^{e}	0.47±0.04^{c}	0.47±0.10^{e,f}	0.94±0.08^{e}
LL2	0.91±0.12^{a,c}	2.24±0.09^{c}	1.10±0.04^{a}	0.41±0.06^{a,c}	0.73±0.10^{b}	1.15±0.10^{b}
LL3	0.62±0.02^{b}	2.15±0.08^{c}	1.07±0.06^{a}	0.28±0.01^{b}	0.80±0.07^{b}	1.08±0.07^{b}

Values are mean±s.d., $n=5$, per group. ^{a,b}Values in same column with different superscripts are significantly different at $P<0.05$.

Table 4.
Effects of an aqueous extract of the leaves of *S. liberica* on the atherogenic indices of normal and alloxan treated rats.

Treatment group	Cardiac risk ratio	Atherogenic coefficient	Atherogenic index of plasma
Normal	1.57±0.11^{a,d}	0.57±0.11^{a,d}	−0.12±0.05^{a}
Test control	3.17±0.15^{c}	2.17±0.15^{c}	0.17±0.05^{c}
Reference	1.53±0.06^{d}	0.53±0.06^{d}	−0.15±0.04^{a}
LLC1	1.75±0.12^{a}	0.75±0.12^{a}	−0.04±0.08^{a,b,e}
LLC2	1.57±0.09^{a,d}	0.57±0.09^{a,d}	−0.14±0.04^{a,b}
LLC3	1.61±0.05^{a,d}	0.61±0.05^{a,d}	−0.08±0.06^{a,b}
LL1	2.09±0.15^{b}	1.09±0.15^{b}	0.08±0.03^{e}
LL2	2.05±0.10^{b}	1.05±0.10^{b}	−0.08±0.07^{b}
LL3	2.01±0.09^{b}	1.01±0.09^{b}	−0.24±0.03^{d}

Values are mean±s.d., $n=5$, per group. ^{a,b}Values in same row with different superscripts are significantly different at $P<0.05$.

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.

The effects of an aqueous extract of the leaves of *S. liberica* on the plasma lipid profiles of normal and alloxan-induced diabetic rats is shown in Table 3. The plasma triglyceride and very low density lipoprotein cholesterol levels of the test control group were significantly higher ($P<0.05$) than those of control, reference, LLC2 and LL3, but not significantly different from those of LLC1, LLC3, LL1 and LL2. The plasma total cholesterol level of the test control group was significantly higher ($P<0.05$) than those of control, reference, LLC1, LLC2, LLC3 and LL1, but not significantly higher than those of LL1 and LL2. The plasma high density lipoprotein cholesterol level of the test control group was significantly lower ($P<0.05$) than those of all the other groups. The plasma low density lipoprotein and non-high density lipoprotein cholesterol levels of the test control group were significantly

Table 5.
Effects of an aqueous extract of *S. liberica* leaves on plasma hepatospecific markers of normal and alloxan treated rats

Treatment group	Magnitude							
	Alkaline phosphatase activity (U/L)	Alanine transaminase activity (U/L)	Aspartate transaminase activity (U/L)	Total bilirubin (μ mol/L)	Conjugated bilirubin (μ mol/L)	Unconjugated bilirubin (μ mol/L)	Unconjugated /conjugated bilirubin ratio	Total protein (mg/dL)
Normal	176.64±2.75^{a}	13.66±0.82^{a}	21.58±0.93^{a}	2.93±0.22^{a,d,e,f}	2.27±0.19^{a,f}	0.66±0.12^{a,e,f,h}	0.30±0.06^{a,e}	50.96±1.84^{a}
Test control	567.87±6.40^{b}	26.61±0.88^{c}	27.05±0.77^{c}	3.64±0.21^{c}	2.71±0.22^{e}	0.93±0.04^{c}	0.35±0.04^{c,d}	59.99±0.92^{c}
Reference	316.19±4.49^{c}	21.00±0.63^{d}	25.92±2.04^{c,d}	3.52±0.08^{c,d}	2.21±0.17^{a,d,f}	1.30±0.14^{d}	0.59±0.11^{e}	57.61±0.93^{d}
LLC1	253.00±7.48^{d}	18.87±1.00^{e,h}	17.48±2.88^{b}	3.13±0.04^{e}	2.33±0.04^{a}	0.81±0.01^{e}	0.35±0.01^{c}	55.01±0.79^{e}
LLC2	212.52±4.93^{e}	15.45±0.52^{f}	16.97±0.83^{b}	2.71±0.02^{f}	1.97±0.02^{b}	0.75±0.02^{f}	0.38±0.01^{d}	55.17±0.95^{e}
LLC3	382.41±7.14^{f}	17.62±0.82^{g,h}	22.05±0.76^{a,e}	3.07±0.05^{e}	2.07±0.02^{f}	1.01±0.05^{g}	0.49±0.02^{b,e}	54.00±1.29^{a,e}
LL1	269.56±2.30^{g}	19.18±0.86^{b,e}	23.26±1.02^{d,e}	2.41±0.06^{b}	1.72±0.05^{e}	0.68±0.01^{h}	0.40±0.01^{b}	53.03±1.22^{a}
LL2	388.24±5.51^{h}	18.16±0.81^{b,g}	24.82±0.66^{d,f}	2.84±0.03^{a}	1.97±0.04^{b,d}	0.87±0.02^{k}	0.44±0.02^{f}	64.03±1.66^{b}
LL3	458.80±4.65^{k}	21.54±0.65^{d}	25.67±0.87^{c,f}	3.61±0.07^{c,d}	2.83±0.07^{c}	0.78±0.01^{b}	0.28±0.01^{a}	56.05±1.88^{d,e}

Values are mean±s.d., n=5, per group. ^{a,b}Values in same column with different superscripts are significantly different at $P<0.05$.

Table 6.
Effects of an aqueous extract of *S. liberica* leaves on the plasma electrolyte profiles of normal and alloxan treated rats.

Treatment group	Concentration						
	Urea (mmol/L)	Blood urea nitrogen (mg/dL)	Calcium (mmol/L)	Sodium (mg/dL)	Potassium (mg/dL)	Chloride (meq/L)	Bicarbonate (meq/L)
Normal	20.70±1.08^{a,b}	58.15±3.03^{a,b}	2.06±0.08^{a,d,e}	129.15±1.82^{a}	5.23±0.07^{a}	98.83±0.84^{a}	18.20±0.84^{a,d}
Test control	28.70±1.01^{c}	80.62±2.82^{c}	1.89±0.06^{c}	148.67±1.75^{c}	5.00±0.07^{b}	84.75±0.43$_{b}$	15.25±0.50^{c}
Reference	12.65±0.52^{d}	35.55±1.45^{d}	1.97±0.06^{a,c,e}	130.00±1.58^{a}	9.20±0.10^{c}	114.66±0.85^{c}	18.00±0.71^{d,e}
LLC1	14.39±0.68^{e}	40.41±1.92^{e}	2.09±0.08^{b,e,g}	127.71±1.20^{a}	5.36±0.06^{d}	96.00±0.71^{d}	18.00±0.71^{d}
LLC2	17.73±0.78^{f}	49.81±2.19^{f}	2.03±0.06^{a,g}	129.33±1.47^{a}	4.90±0.07^{b}	102.67±0.41^{e}	17.00±0.71^{a}
LLC3	13.68±0.55^{e}	38.43±1.54^{e}	2.07±0.06^{b,e}	139.40±1.95^{d}	6.68±0.08^{e}	100.60±0.55^{f}	18.00±0.71^{d}
LL1	20.65±0.38^{a}	58.00±1.08^{a}	2.12±0.09^{b,e}	134.33±2.68^{b,d}	5.60±0.07^{f}	88.67±0.41^{g}	16.67±0.41^{e}
LL2	21.17±0.93^{a}	59.46±2.62^{a}	1.96±0.06^{a}	130.00±2.45^{a,b}	6.00±0.07^{g}	98.00±0.71^{a}	21.67±0.41^{b}
LL3	19.00±0.59^{b}	53.37±1.65^{b}	2.11±0.07^{b,d}	129.00±2.55^{a}	5.53±0.04^{h}	97.50±0.50^{h}	19.00±0.71^{d}

Values are mean±s.d., n=5, per group. ^{a,b}Values in the same column with different superscripts are significantly different at $P<0.05$.

Table 7.
Effects of an aqueous extract of the leaves of *S. liberica* on ocular markers of oxidative stress in normal and alloxan treated rats.

Treatment group	Ascorbic acid content (μ mole/g protein)	Malondialdehyde content (μ mol/g protein)	Catalase activity (Units/mg protein)	Superoxide dismutase activity (Units/mg protein)
Normal	11.233±0.474^{a,e}	0.091±0.001^{a}	20.595±0.855^{a,f,g}	0.196±0.001^{a}
Test control	8.295±0.011^{c}	0.098±0.001^{c}	18.843±0.190^{e}	0.157±0.002^{b}
Reference	8.724±0.107^{d}	0.074±0.001^{d}	23.425±0.533^{d}	0.182±0.001^{c}
LLC1	11.666±0.312^{e}	0.090±0.001^{a}	22.298±0.576^{d}	0.193±0.001^{d}
LLC2	14.750±0.238^{f}	0.086±0.003^{e}	26.453±0.045^{e}	0.209±0.000^{e}
LLC3	12.582±0.096^{g}	0.079±0.002^{f}	21.220±0.238^{f}	0.193±0.000^{d}
LL1	9.889±0.073^{b}	0.076±0.001^{d}	19.628±0.076^{g}	0.185±0.001^{f}
LL2	12.462±0.022^{g}	0.086±0.001^{e}	20.537±0.050^{a}	0.203±0.000^{g}
LL3	10.744±0.073^{a}	0.030±0.000^{b}	24.933±0.084^{b}	0.212±0.000^{h}

Values are mean±s.d., n=5, per group. ^{a,b}Values in the same column with different superscripts are significantly different at $P<0.05$.

Table 8.

Effects of an aqueous extract of the leaves of *S. liberica* on the hematological indices of normal and alloxan treated rats.

Parameter	Magnitude								
	Normal	Test control	Reference	LLC1	LLC2	LLC3	LL1	LL2	LL3
Hematocrit (%)	34.4±0.9^{a}	31.0±1.0^{c}	38.8±0.8^{d}	45.8±0.4^{e}	45.8±0.8^{b,e}	47.5±0.5^{f}	46.7±0.4^{b}	46.0±0.7^{b,e}	46.7±0.4^{b,f}
Hemoglobin concentration (g/dL)	12.0±0.1^{a}	11.4±0.2^{b}	12.3±0.4^{a}	13.1±0.1^{c}	13.9±0.1^{d}	14.6±0.1^{e}	14.3±0.0^{f}	14.1±0.0^{g}	13.8±0.1^{d}
Red cell count (×10^{9} cells/L)	5.7±0.4^{a,c}	5.5±0.0^{c}	6.7±0.3^{d}	4.3±0.1^{e}	4.3±0.1^{e}	6.0±0.2^{a}	4.3±0.4^{e}	5.1±0.1^{b}	4.2±0.1^{e}
Total white cell count (×10^{9} cells/L)	9.7±0.4^{a}	12.0±0.3^{c}	10.2±0.2^{d}	4.4±0.1^{e}	4.5±0.1^{e}	8.4±0.6^{b}	7.8±0.1^{b}	7.0±0.1^{f}	7.2±0.5^{b,f}
Neutrophils count (%)	5.3±0.4^{a}	3.7±0.5^{b}	5.5±0.5^{a}	52.8±0.9^{c}	53.0±1.2^{c}	42.7±0.9^{d}	33.3±0.8^{e}	30.3±0.4^{f}	31.0±0.7^{f}
Lymphocytes count (%)	81.7±0.5^{a}	83.3±0.5^{b}	77.5±0.5^{c}	44.8±0.8^{d}	42.3±0.8^{e}	51.6±0.6^{f}	65.0±1.4^{g}	66.7±0.9^{h}	68.0±0.7^{h}
Monocytes count (%)	12.8±1.1^{a}	12.8±0.8^{a}	15.6±1.1^{b}	1.8±0.3^{c}	2.5±0.1^{d}	5.2±0.1^{e}	1.6±0.1^{c}	1.3±0.1^{f}	0.7±0.1^{g}
Eosinophils count (%)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.4±0.1^{c,e}	0.8±0.2^{b,d,f}	0.5±0.1^{d,e}	0.0±0.0^{a}	1.0±0.1^{b}	0.3±0.1^{c,f}
Basophils count (%)	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.4±0.0^{b}	0.2±0.1^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.4±0.0^{b}	0.0±0.0^{a}
Mean cell volume (fL)	60.1±3.8^{a}	56.4±1.6^{c}	57.9±2.1^{ac}	107.1±3.5^{d}	107.1±2.5^{d}	79.4±2.5^{e}	108.5±10.4^{d}	89.7±3.2^{b}	110.3±3.7^{d}
Mean cell haemoglobin concentration (g/dL)	34.8±1.1^{a}	36.7±1.5^{a}	31.8±0.4^{c}	28.6±0.4^{d}	30.4±0.6^{b,e}	30.7±0.4^{b}	30.6±0.3^{b}	30.7±0.4^{b}	29.6±0.2^{e}
Mean cell haemoglobin (pg/cell)	2.1±0.2^{a}	2.1±0.0^{a}	1.8±0.1^{c}	3.1±0.1^{d}	3.3±0.1^{e}	2.4±0.1^{f}	3.3±0.3^{d,e}	2.8±0.1^{b}	3.3±0.1^{e}

Values are mean±s.d., n=5, per group. ^{a,b}Values in same row with different superscripts are significantly different at P<0.05.

higher (P<0.05) than those of all the other groups.

The effects of an aqueous extract of the leaves of *S. liberica* on the atherogenic indices of normal and alloxan-induced diabetic rats is shown in Table 4. All the atherogenic indices (cardiac risk ratio, atherogenic coefficient and atherogenic index of plasma) of the test control group were significantly higher (P<0.05) than those of all the other groups.

The effects of an aqueous extract of the leaves of *S. liberica* on the levels of plasma markers of liver integrity and function in normal and alloxan-induced diabetic rats is given in Table 5. The plasma alkaline phosphatase and alanine transaminase activities of the test control group were significantly higher (P<0.05) than those of all the other groups. The plasma aspartate transaminase activity of the test control group was significantly higher (P<0.05) than those of control, LLC1, LLC2, LLC3, LL1 and LL2, but not significantly higher than those of reference and LLC3. The plasma total bilirubin level of the test control group was significantly higher (P<0.05) than all the other those groups, except reference and LL3. The plasma conjugated bilirubin level of the test control group was significantly higher (P<0.05) than all the other groups, except LL3. The plasma unconjugated bilirubin level of the test control group was significantly higher (P<0.05) than those of control, LLC1, LLC2, LL1, LL2 and LL3, but significantly lower (P<0.05) than reference and LLC3. Plasma unconjugated/conjugated bilirubin level of the test control group was significantly lower (P<0.05) than than those of reference, LLC3, LL1 and LL2, and significantly higher (P<0.05) than that of LL3, but not significantly different from those of control, LLC1 and LLC2. The plasma total protein concentration of the test control group was significantly lower (P<0.05) than that of LL2, but significantly higher (P<0.05) than those of all the other groups.

The effects of an aqueous extract of the leaves of *S. liberica* on the plasma electrolyte profiles of normal and alloxan-induced diabetic rats is shown in Table 6. The urea, blood urea nitrogen and sodium levels of the test control group were significantly higher (P<0.05) than those of all the other groups. The plasma bicarbonate and chloride levels of the test control group were significantly lower (P<0.05) than those of all the other groups. The plasma calcium level of the test control group was significantly lower (P<0.05) than those of all the other groups except reference. The plasma potassium level of the test control group was significantly lower (P<0.05) than those of all the other groups except LLC2.

Table 7 shows the effects of an aqueous extract of the leaves of *S. liberica* on ocular markers of oxidative stress in normal and alloxan-induced diabetic rats. The ocular ascorbic acid content of the test control group was significantly lower (P<0.05) than those of all the other groups. The ocular malondialdehyde content of the test control group was significantly higher (P<0.05) than those of all the other groups. The ocular catalase and superoxide dismutase activities of the test control group was significantly lower (P<0.05) than those of all the other groups.

The effects of an aqueous extract of the leaves of *S. liberica* on the hematological indices of normal and alloxan-induced diabetic rats is presented in Table 8. The hematocrit, hemoglobin concentration and neutrophil count of the test control group was significantly lower (P<0.05) than those of all the other groups. The red cell count of the test control group was significantly lower (P<0.05) than those of reference and LLC3, and significantly higher (P<0.05) than those of LLC1, LLC2, LL1, LL2 and LL3, but not significantly different from control. The total white cell and lymphocyte counts of the test control group were significantly higher (P<0.05) than those of all the other groups. The monocyte count of the test control group was significantly higher (P<0.05) than those of all the other groups, except the control and reference that were not significantly different and significantly

higher ($P<0.05$) respectively. The mean cell hemoglobin concentration of the test control group was significantly higher ($P<0.05$) than those of all the other groups, except the control that was not significantly different. The mean cell hemoglobin of the test control group was significantly lower ($P<0.05$) than those of all the other groups, except the control and reference that were not significantly different and significantly lower ($P<0.05$) respectively. The mean cell volume of the test control group was significantly lower ($P<0.05$) than those of all the other groups, except the reference that was not significantly different. There were no significant differences in the eosinophil and basophil counts of all the groups.

4. Discussion

Diabetes mellitus induced by alloxan, is usually characterized by decreased insulin level, hyperglycemia, elevated triglycerides and total cholesterol, and decreased high density lipoprotein[31]. The high percentage reduction in plasma glucose levels, produced by the extract in this study, supports the use of the plant in the management of diabetes mellitus. The hypoglycemic effect of the extract may have been produced by the flavonoids, saponins and tannins present in the leaves[5,7]. The flavonoids, saponins and tannins are families of compounds with established hypoglycemic activity[32-37]. Thus, anyone or a combination of some or all of the above mentioned components could have been responsible for the hypoglycemic effect of the extract, observed in this study.

Elevated levels of plasma triglyceride, is both an independent and synergistic risk factor for cardiovascular diseases[38-40]. It is often associated with hypertension[41,42], abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus[40,43,44]. The treatments significantly reduced plasma levels of triglycerides. This effect may have been mediated by the flavonoid[5] and vitamin C[6] contents of the leaves; since flavonoids[45] and vitamin C[46-48] are known to decrease plasma triglyceride levels.

The administration of the extract produced significantly higher plasma HDL cholesterol levels. This portends reduction of cardiovascular risk; since increases in plasma HDL cholesterol concentration are known to decrease cardiovascular risk[49,50].

Elevated plasma total cholesterol level is a recognized and well-established risk factor for developing atherosclerosis and other cardiovascular diseases[51], and is found in diabetes mellitus. Therefore, a reduction in plasma total cholesterol level reduces the risk of cardiovascular diseases. Consequently, the dose dependent reduction in plasma total cholesterol levels produced by the extract, connotes the ability of the extract to protect against cardiovascular complications.

Elevated levels of plasma VLDL cholesterol is a risk factor for cardiovascular disease[51,52] and often found in the diabetic[18,44,50] and obese[53] individuals. In this study, a significantly lower plasma VLDL cholesterol levels was observed in the treated animals compared to control. This is an indication of the cardio-protective potential of the extract.

Raised plasma level of LDL cholesterol is a risk factor for cardiovascular disease[51,52] and often accompanies hypertension[54,42] and diabetes mellitus. Conversely, reductions in plasma LDL cholesterol have been considered to reduce risk of coronary heart disease[44,50]. In this study, a significantly lowered plasma LDL cholesterol levels was observed in the animals given the extract, suggesting the likely cardio-protective effect of the extract.

Many studies have shown that non-HDL cholesterol is a better predictor of cardiovascular disease risk than LDL cholesterol[18,55,56]. Therefore, the significantly lower plasma non HDL cholesterol observed in the treated diabetic groups is another indicator of the ability of the extract to reduce cardiovascular risk.

This hypocholesterolemic effect of the extract may be due to the leaves' content of flavonoids, saponins and tannins[5,7], which are known to have cholesterol lowering and atheroprotective activities[35,57,58,59]. Again, 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.

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[38,39,60,61]. Low atherogenic indices are protective against coronary heart disease[61]. This again highlights the ability of the extract to protect against cardiovascular complications in the diabetic.

These results show that the extract had a dose dependent positive effect on the integrity and function of the liver and kidney of the diabetic rats. It improved the lowered plasma calcium level produced by the diabetic condition. The extract may have achieved this by affecting parathyroid hormone secretion. This hormone increases the renal tubular re-absorption of calcium, promotes intestinal calcium absorption[62,63]. The significance of this cannot be overemphasized. Many neuromuscular and other cellular functions are dependent on the maintenance of the ionized calcium concentration in the extracellular fluid[63]. Calcium fluxes are also important mediators of hormonal effects on target organs through several intracellular signaling pathways, such as phosphoinositide and cyclic adenosine monophosphate systems[63,64].

Reduced erythrocyte and plasma potassium concentrations have been associated with glucose intolerance. Potassium depletion causes glucose intolerance, which is associated with impaired insulin secretion[65]. Therefore, the alleviation of the diabetes induced reduction in plasma potassium

levels portends ability of the extract to improve insulin activity. The treatment also alleviated the diabetes induced metabolic acidosis in the test animals.

Induction of diabetes in rats with alloxan usually results in an increase in thiobarbituric acid reactive substances, an indirect evidence of intensified free-radical production[66]. Increased levels of these thiobarbituric acid reactants are consistently observed in diabetes[67-74]. Increases in blood and tissue levels of thiobarbituric acid-reactive substances, mainly malondialdehyde, are very reliable indices of oxidative stress and lipid peroxidation[69,75]. Thus the profiles of malondialdehyde seen in the test rats clearly indicate that the extract protects against oxidative stress in ocular tissues. This antioxidant protective effect may be due to the presence of flavonoids and tannins in the leaves. Both families of compounds have established antioxidant activities[35,36,45,57,59].

Reduced levels and altered metabolic turnover of ascorbic acid have been reported in diabetic patients[69,74]. According to Samuel *et al*[74], the high extracellular glucose concentration in the diabetic impairs the cellular uptake of ascorbic acid, and accentuates the problems which are associated with its deficiency. Studies show that ascorbic acid protects the lens and other tissues of the eye from light damage[76,77]. So, the high ocular ascorbic acid levels caused by the extract, portends a consolidation of antioxidant status of the eyes, hence protection of its tissues from free radical damage. This correlates with the observed reduction in the level of lipid peroxidation. This increase in ascorbic acid content of the eyes of the test animals may have resulted from the high ascorbic acid content of the leaves[6].

In diabetics, reduced activities of the antioxidant enzyme systems often results from the progressive glycation of the enzymatic proteins[68,69,74]. Many studies have reported variations in the levels of antioxidants in the diabetic patients[68,74]. In this study, the treatment improved the ocular activities of catalase and superoxide dismutase of the animals.

The extract had a positive effect on the hemopoietic system of the test rats. It significantly increased the hematocrit, hemoglobin concentration, neutrophil and monocyte counts while decreasing total white cell and lymphocyte counts. White blood cells play important roles in the destabilization of coronary artery plaques at the onset of acute coronary syndrome[78-80]. Nevertheless, an elevated white blood cell count in peripheral blood is a known risk factor of coronary artery disease[81]. Therefore, the lower total white blood cell count, observed in the test rats, is an indication of the ability of the extract to protect against diabetes induced increases in total white cell count. It also means reduction of the risk of coronary artery disease. The decreased white cell count may have been produced by the immune-modulating activity of saponins[35,82] and tannins[83], present in the leaves of *S. liberica*.

This study showed that the extract was hypoglycemic, positively affected the hemopoietic 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. All of these, highlight the cardio-protective potential of the leaves of *S. liberica*, and support its use in traditional health care practices for the management of diabetes mellitus.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Evans WC. A taxonomic approach to the study of medicinal plants and animal-derived drugs. In: Evans WC, editor. *Trease and Evans pharmacognosy*, 15th edn. Elsevier: India; 2005, p. 15-40.

[2] Reed DW. Sansevieria-Identification and culture. *Louisiana Soc Horticult Res* 1978; **14**(2): 30-77.

[3] United States Department of Agriculture, Agricultural Research Service, Germplasm Resources Information Network (GRIN). GRIN taxonomy for plants [database on the Internet]. c2008 [cited 2008 July 23]. Available from: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?33057

[4] Osabohien E, Egboh SHO. Utilization of bowstring hemp fiber as a filler in natural rubber compounds. *J Appl Polymer Sci* 2008: **107**: 210-214.

[5] Ikewuchi CC, Ikewuchi CJ, Ayalogu OE, Onyeike NE. Proximate and phytochemical profile of *Sansevieria liberica* Gérôme and Labroy. *J Appl Sci Environ Mgt* 2010; **14**(2): 103-106.

[6] Ikewuchi CC, Ikewuchi JC. Amino acid, mineral, and vitamin composition of *Sansevieria liberica* Gérôme and Labroy. *Pac J Sci Technol* 2009; **10**(1): 477-482.

[7] Ikewuchi CC, Ayalogu EO, Onyeike EN, Ikewuchi JC. Study on the alkaloid, allicin, glycoside and saponin composition of the leaves of *Sansevieria liberica* Gérôme and Labroy by gas chromatography. *Pac J Sci Technol* 2011; **12**(1): 367-373.

[8] Osabohien E. Effects of epoxidation on the thermal conductivity and equilibrium swelling properties of bowstring hemp fibre natural rubber composites. *Adv Nat Appl Sci Res* 2009; **7**: 169-176.

[9] Gill LS. *Ethnomedical uses of plants in Nigeria*. Nigeria: Uniben Press, Benin City; 1992.

[10] Amida MB, Yemitan OK, Adeyemi OO. Toxicological assessment of the aqueous root extract of *Sanseviera liberica* Gerome and Labroy (Agavaceae). *J Ethnopharmacol* 2007; **113**(1): 171-175.

[11] Adeyemi OO, Akindele AJ, Ogunleye EA. Evaluation of the antidiarrhoeal effect of Sanseviera liberica Gerome and Labroy (Agavaceae) root extract. *J Ethnopharmacol* 2009; **123**(3): 459-463.

[12] Ikewuchi CC, Ayalogu EO, Onyeike EN, Ikewuchi JC. Effect of

aqueous extract of the leaves of *Sansevieria liberica* Gérôme and Labroy on blood pressure indices and pulse rates of sub-chronic salt-loaded rats. *J Nat Remed* 2011; **11**(2): 30-38.

[13]Ikewuchi CC, Ikewuchi JC, Onyeike EN, Ayalogu EO. Effect of *Sansevieria liberica* Gérôme and Labroy on plasma chemistry and hematological indices of salt-loaded rats. *Res J Sci Technol* 2010; **2**(5): 110-114.

[14]Radwan MA. Enhancement of absorption of insulin-loaded polyisobutylcyanoacrylate nanospheres by sodium cholate after oral and subcutaneous administration in diabetic rats. *Drug Develop Ind Pharm* 2001; **27**(9): 383-391.

[15]Burcelin R, Eddouks M, Maury J, Kande J, Assan R, Girard J. Excessive glucose production, rather than insulin resistance, account for hyperglycemia in recent onset streptozocin-diabetic rats. *Diabetologia* 1995; **385**(3): 283-290.

[16]Jyoti M, Vihas TV, Ravikumar A, Sarita G. Glucose lowering effect of aqueous extract of *Enicostemma littorale* Blume in diabetes: a possible mechanism of action. *J Ethnopharmacol* 2002; **81**: 317-320.

[17]Friedewald WT, Levy RI, Friedrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**(6): 499-502.

[18]Brunzell JD, Davidson M, Furberg CD, Goldberg RD, Howard BV, Stein JH, et al. Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. *J Am Coll Cardiol* 2008; **51**(15): 1512-1524.

[19]Ikewuchi JC, Ikewuchi CC. Alteration of plasma lipid profile and atherogenic indices of cholesterol loaded rats by *Tridax procumbens* Linn: Implications for the management of obesity and cardiovascular diseases. *Biokemistri* 2009; **21**(2): 95-99.

[20]Ikewuchi JC, Ikewuchi CC. Alteration of plasma lipid profiles and atherogenic indices by *Stachytarpheta jamaicensis* L. (Vahl). *Biokemistri* 2009; **21**(2): 71-77.

[21]Ikewuchi JC, Ikewuchi CC. Hypocholesterolaemic effect of aqueous extract of Acalypha wilkesiana 'Godseffiana' Muell Arg on rats fed egg yolk supplemented diet: Implications for cardiovascular risk management. *Res J Sci Technol* 2010; **2**(4): 78-81.

[22]AOAC International. *Official methods of analysis of the AOAC* (W. Horwitiz, editor), 18th edn. Washington D.C., USA: AOAC International; 2006.

[23]Baginsky ES, Marie SS, Clark WL, Zak B. Direct microdetermination of calcium. *Clin Chim Acta* 1973; **46**: 49-54.

[24]Cheesbrough M. *District laboratory practice in tropical countries, Part* 1. UK: Cambridge University Press; 2006.

[25]Hunter FE, Gebicki JM, Hoffstein PE, Weinstein J, Scott A. Swelling and lysis of rat liver mitochondria induced by ferrous ions. *J Biol Chem* 1963; **238**: 828-835.

[26]Gutteridge JMC, Wilkins C. Copper-dependent hydroxyl radical damage to ascorbic acid. Formation of a thiobarbituric acid reactive products. *FEBS Letters* 1982; **137**: 327-340.

[27]Ikewuchi JC, Ikewuchi CC. Iodometric determination of the ascorbic acid (vitamin C) content of some fruits consumed in a university community in Nigeria. *Global J Pure Appl Sci* 2011; **17**(1): 47-49.

[28]Ikewuchi JC, Onyeike EN, Uwakwe AA, Ikewuchi CC. Effect of aqueous extract of the leaves of *Acalypha wilkesiana* 'Godseffiana' Muell Arg (Euphorbiaceae) on the hematology, plasma biochemistry and ocular indices of oxidative stress in alloxan induced diabetic rats. *J Ethnopharmacol* 2011; **137**(3): 1415-1424.

[29]Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Biol Chem* 1952; **195**: 130-140.

[30]Misra HP, Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and a single assay for superoxide dismutase. *J Biol Chem* 1989; **247**: 3170-3175.

[31]Hemalatha R. Anti-hepatotoxic and anti-oxidant defense potential of *Tridax procumbens*. *Internl J Green Pharm* 2008; **2**(3): 164-169.

[32]Liu F, Kim J, Li Y, Liu X, Li J, Chen X. An extract of *Lagerstroemia speciosa* L. has insulin-like glucose uptake-stimulatory and adipocyte differentiation-inhibitory activities in 3T3-L1 cells. *J Nutr* 2001; **131**: 2242-2247.

[33]Liu X, Kim J-K, Li Y, Li J, Liu F, Chen X. Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. *J Nutr* 2005; **135**: 165-171.

[34]Muthusamy VS, Anand S, Sangeetha KN, Sujatha S, Lakshmi BABS. Tannins present in *Cichorium intybus* enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. *Chem Biol Interact* 2008; **174**: 69-78.

[35]Soetan KO. Pharmacological and other beneficial effects of antinutritional factors in plants-A review. *Afr J Biotechnol* 2008; **7**(25): 4713-4721.

[36]Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: A review. *Trop J Pharmaceut Res* 2008; **7**(3): 1089-1099.

[37]Pereira DM, Valentão P, Pereira JA, Andrade PB. Phenolics: From chemistry to biology. *Molecules* 2009; **14**: 2202-2211.

[38]Dobiášová M. Atherogenic index of plasma [log(triglyceride/HDL-Cholesterol)]: theoretical and practical implications. *Clin Chem* 2004; **50**: 1113-1115.

[39]Martirosyan DM, Miroshnichenko LA, Kulokawa SN, Pogojeva AV, Zoloedov VI. Amaranth oil application for heart disease and hypertension. *Lipids Health Dis* 2007; **6**: 1

[40]McBride PE. Triglycerides and risk for coronary heart disease. *J Am Med Assoc* 2007; **298**: 336-338

[41]Lopes FH, Bernardes Silva H, Soares JA, Filho B, Consolim-Colombo FM, Giorgi DMA, et al. Lipid metabolism alterations in normotensive subjects with positive family history of hypertension. *Hypertension* 1997; **30**: 629-631.

[42]Zicha J, Kunes J, Devynck MA. Abnormalities of membrane function and lipid metabolism in hypertension: a review. *Am J Hypertens* 1999; **12**: 315-331.

[43]Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, et al. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabet Care* 2002; **25**: 148-198.

[44]Shen GX. Lipid disorders in diabetes mellitus and current management. *Curr Pharmaceut Anal* 2007; **3**: 17-24.

[45]Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol Rev* 2000; **52**(4): 673–751.

[46]Houston MC. The role of vascular biology, nutrition and nutraceuticals in the prevention and treatment of hypertension. *J Am Nutraceut Assoc* 2002; (Suppl. 1): 5–71.

[47]Houston MC. Nutraceuticals, vitamins, antioxidants, and minerals in the prevention and treatment of hypertension. *Prog Cardiovasc Dis* 2005; **47**(6): 396–449.

[48]Houston MC. Treatment of hypertension with nutraceuticals, vitamins, antioxidants and minerals. *Expert Rev Cardiovasc Ther* 2007; **5**(4): 681–691.

[49]Assmann G, Gotto AM Jr. HDL cholesterol and protective factors in atherosclerosis. *Circulation* 2004; **109**(suppl III): III8–III14.

[50]Rang HP, Dale MM, Ritter JM, Moore PK. *Pharmacology*, 5th edn. India: Elsevier; 2005.

[51]Ademuyiwa O, Ugbaja RN, Idumebor F, Adebawo O. Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta, Nigeria. *Lipids Health Dis* 2005; **4**: 19.

[52]Lichtennstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franklin B, et al. Diet and lifestyle recommendations revision 2006. A Scientific Statement from the American Heart Association Nutrition Committee. *Circulation* 2006; **114**(1): 82–96.

[53]Krauss RM, Blanche PJ, Rawlings RS, Fernstrom HS, Williams PT. Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. *Am J Clin Nutr* 2006; **83**(6): 1025–1031.

[54]Shepherd J. Identifying patients at risk for coronary heart disease: Treatment implications. *Eur Heart J* 1998; **19**: 1776–1783.

[55]Liu J, Sempos C, Donahue R, Dorn J, Trevisan M, Grundy SM. Joint distribution of non–HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes. *Diabet Care* 2005; **28**(8): 1916–1921.

[56]Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non–high density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation* 2005; **112**(22): 3375–3383.

[57]Dillard CJ, German JB. Phytochemicals: nutraceuticals and human health. *J Sci Food Agric* 2000; **80**(12): 1744–1756.

[58]Berhow MA, Cantrell CL, Duval SM, Dobbins TA, Mavnes J, Vaughn SF. Analysis and quantitative determination of group B saponins in processed soybean product. *Phytochem Anal* 2002; **13**: 343–348.

[59]Basu SK, Thomas JE, Acharya SN. Prospects for growth in global nutraceutical and functional food markets: A Canadian perspective. *Aust J Basic Appl Sci* 2007; **1**(4): 637–649

[60]Brehm A, Pfeiler G, Pacini G, Vierhapper H, Roden M. Relationship between serum lipoprotein ratios and insulin resistance in obesity. *Clin Chem* 2004; **50**(12): 2316–2322.

[61]Usoro CAO, Adikwuru CC, Usoro IN, Nsonwu AC. Lipid profile of postmenopausal women in Calabar, Nigeria. *Pak J Nutr* 2006; **5**(1): 79–82.

[62]Brown EM, Hebert SC. Calcium–receptor–regulated parathyroid and renal function. *Bone* 1997; **20**: 303–309.

[63]Crook MA. *Clinical chemistry and metabolic medicine*, 7th edn. London: Holder Arnold; 2006.

[64]FAO. *Vitamin and mineral requirements in human nutrition,* 2nd edn. Bangkok: FAO/WHO Expert Consultation; 2004.

[65]Rowe JW, Tobin JD, Rosa RM, Andres R. Effect of experimental potassium deficiency on glucose and insulin metabolism. *Metabolism* 1980; **29**(6): 498–502.

[66]Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: A review. *J Biochem Mol Toxicol* 2003; **17**(1): 24–38.

[67]Wolff SP. Diabetes mellitus and free radicals: Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *Brit Med Bull* 1993; **49**(3): 642–432.

[68]Hartnett ME, Stratton RD, Browne RW, Rosner BA, Lanham RJ, Armstrong D. Serum markers of oxidative stress and severity of diabetic retinopathy. *Diabet Care* 2000; **23**(2): 234–240.

[69]Atalay M, Laaksonen DE. Diabetes, oxidative stress and physical exercise. *J Sports Sci Med* 2002; **1**: 1–14.

[70]Rajdl D, Racek J, Steinerová A, Novotný Z, Stožický F, Trefil L, et al. Markers of oxidative stress in diabetic mothers and their infants during delivery. *Physiol Res* 2005; **54**: 429–436.

[71]Okafor OY, Erukainure OL, Ajiboye JA, Adejobi RO, Owolabi FO, Kosoko SB. Modulatory effect of pineapple peel extract on lipid peroxidation, catalase activity and hepatic biomarker levels in blood plasma of alcohol–induced oxidative stressed rats. *Asian Pac J Trop Biomed* 2011; **1**(1): 12–14.

[72]Rao PV, Sujana P, Vijayakanth T, Naidu MD. *Rhinacanthus nasutus* – Its protective role in oxidative stress and antioxidant status in streptozotocin induced diabetic rats. *Asian Pac J Trop Dis* 2012; **2**(4): 327–330.

[73]Behera S, Mangaraj M, Mohapatra PC. Diagnostic efficacy of ischemia modified albumin and its correlation with lipid profile, oxidative stress in acute myocardial infarct patients on admission. *Asian Pac J Trop Dis* 2012; **2**(1): 62–65.

[74]Samuel VT, Jayaprakash Murthy DS, Dattatreya K, Babu PS, Johncy SS. Impaired antioxidant defence mechanism in diabetic retinopathy. *J Clin Diag Res [serial online]* 2010; **4**(6): 3430–3436.

[75]Imai K, Aimoto T, Sato M, Kimura R. Antioxidative effect of protoporphyrin on lipid peroxidation in tissue homogenates of intravenously administered rats. *J Pharmacobio–Dyn* 1991; **14**(1): 20–24.

[76]Varma SD. Scientific basis of medical therapy of cataracts by antioxidants. *Am J Clin Nutr* 1991; **53**: 335S–345S.

[77]Taylor A. Relationship between nutrition and oxidation. *J Am Coll Nutr* 1993; **12**(2): 138–146.

[78]Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes: Implications for plaque rupture. *Circulation* 1994; **90**(2): 775–778.

[79]Van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994; **89**(1): 36–44.

[80]Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 2001; **104**(3): 365–372.

[81]Takeda Y, Suzuki S, Fukutomi T, Kondo H, Sugiura M, Suzumura H, et al. Elevated white blood cell count as a risk factor of coronary artery disease: Inconsistency between forms of the disease. *Jpn Heart J* 2003; **44**(2): 201–211.

[82]European Food Safety Authority. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Saponins in *Madhuca longifolia* L. as undesirable substances in animal feed. *EFSA J* 2009; **979**: 1–36.

[83]Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. *Crit Rev Food Sci Nutr* 1998; **38**: 421–464.