Antidiabetic and antioxidant activities of the methanol leaf extract of Vernonia amygdalina in alloxan-induced diabetes in Wistar rats

The methanolic leaf extract of Vernonia amygdalina (MLVA) was assessed to evaluate its antidiabetic potential in rats. Diabetes was induced in male Wistar rats by the administration of alloxan monohydrate at 100 mg/kg of body weight. After 48 h, rats with fasting blood glucose levels of 200 mg/dL and above were considered diabetic and used for the study. The experimental animals were grouped into five groups (A–E) of 10 animals each. Group A rats were non-diabetic normal control, Group B consisted of diabetic control rats that received no treatment, groups C, D and E rats were diabetic rats but treated with glibenclamide, 200 and 400 mg/kg doses of MLVA respectively. Blood samples were collected at days 14 and 28 after induction for haematological and serum biochemical indices such as triglycerides, LDL, cholesterol etc. The intestine was collected and intestinal homogenate was prepared for the antioxidant studies. The extract at 200 mg/kg and 400 mg/kg doses significantly (p < 0.05) reduced blood glucose levels in extract-treated diabetic rats and also significantly increased weight gain in these rats. Most haematological parameters in treated rats experienced, while platelets and neutrophils were decreased. Biochemical indices measured were reduced in MLVA-treated groups compared with diabetic control. Treatment with MLVA also produced significant (p < 0.05) decrease in markers of oxidative stress but increased levels of enzymic and non-enzymic antioxidant markers in intestinal homogenates of treated groups compared with diabetic control. This study showed that V. amygdalina has antihyperglycaemic and in vivo antioxidant effects.

Introduction

Diabetes mellitus is a disorder of carbohydrate, protein and fat metabolism and can represent absolute insulin deficiency, impaired release of insulin by the pancreatic β cells, inadequate or defective insulin receptors or production of inactive insulin (Porth 1998). Diabetes mellitus is still one of the most important causes of death and disability in both developed and developing countries. According to the report by World Health Organization (WHO 2015), 9% of adults in the world suffer from diabetes and this disease will be the seventh leading cause of death in 2030. There is substantial evidence that diabetes is also epidemic in many low-income and middle-income countries (WHO 1994). From the African continent counts, approximately 13.6 million people are diabetic, with Nigeria having the highest number of people with diabetes (about 1 218 000 people affected). Nigeria also has the highest number of people (estimated at 3.85 million) with impaired glucose tolerance (International Diabetes Federation 2011).

There are two predominant forms of diabetes mellitus, type 1 and type 2. Type 1 diabetes mellitus is characterised by absolute insulin deficiency that results from an autoimmune destruction of pancreatic islet cells; therefore, it is referred to as insulin-dependent diabetes mellitus (Achenbach et al. 2005). Type 2 diabetes mellitus in contrast is a disease of dual defects of insulin resistance and relative insulin deficiency. It develops from an initial period of insulin resistance and relatively preserved insulin secretion as the pancreas attempts to maintain eu glycaemia. Pancreatic β-cell function ultimately falters and is no longer able to meet peripheral demands. Insulin levels decline and hyperglycaemia ensues (Reaven 2004). Based on existing data, type 2 diabetes accounts for around 90%–95% of all diagnosed cases of diabetes mellitus worldwide (Mohan & Anbalagan 2013; Shaw, Sicree & Zimmet 2010; Uma & Sudarsanam 2012). At present, only insulin and oral antihyperglycaemic drugs are available for the management of diabetes (Lorenzati et al. 2010). Although the currently available drugs are useful in controlling early onset complications of diabetes, serious late onset complications appear in a large number of patients (Tzoulaki et al. 2009).
Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes (Johnson et al. 2015). Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have access to conventional antidiabetic therapies (Achaya & Shrivastava 2004; Grover, Yadav & Vats 2002). In developed countries, the use of antidiabetic herbal remedies has been on the decline since the introduction of insulin and synthetic oral hypoglycaemic drugs during the early part of the 20th century. However, recently in the developed countries, there has been the resurgence of interest in medicinal plants that exhibit hypoglycaemic property (Haq 2004). The renewed interest in herbal antidiabetic remedies in developed countries is believed to be motivated by several factors that include adverse reactions, high secondary failure rates and cost of conventional synthetic antidiabetic remedies (Gurib-Fakim 2006). The World Health Organization has recommended the use of medicinal plants for the management of diabetes and further encouraged the expansion of the frontiers of scientific evaluation of hypoglycaemic properties of diverse plant species (Dirks 2004). Consequently, estimates show that over 70% of the global population use traditional medicine for the management and alleviation of diabetes and its complications (Haq 2004; Remuzzi, Macia & Ruggerenti 2006).

Vernonia amygdalina, a woody shrub that can grow up to 5 m tall, belonging to the family of Asteraceae, is native to Nigeria (West Africa) and is widely grown in Africa (Farombi & Owoeye 2011). Vernonia amygdalina is also found in Asia, and is especially common in Singapore and Malaysia (Yeap et al. 2010). The leaves have a characteristic odour and bitter taste, explaining its common English name of ‘bitter leaf’. Vernonia amygdalina has been shown to possess diverse therapeutic effects such as antimalarial, antimicrobial (antibacterial, antifungal, antiplasmodial, etc.), antidiabetic and anticancer effects (Ijeh & Ejike 2011; Yeap et al. 2010).

Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. In the search for alternative herbal treatment for diabetes, this study was conducted to evaluate the antidiabetic and antioxidant properties of the methanol leaf extract of Vernonia amygdalina (MLVA) in alloxan-induced diabetic rats.

Research methods and design

Plant collection and preparation of extract

Fresh leaves of V. amygdalina were collected from the community around the University of Ibadan, Ibadan, Nigeria. The leaves were dried under shade for about 14 days after which they were ground to powder using an electric blender. The powdered material weighing about 600 g was soaked in 2.5 L of methanol and shaken vigorously. The sample was then left for 72 h with intermittent shaking. After 72 h, the mixture was filtered using a Buckner funnel and Whatman No. 1 filtered paper. The extract was further concentrated using a rotary evaporator. The weight of the resulting extract was 32.4 g.

Experimental animals

A total of 70 male albino rats (150 g–220 g) of the Wistar strain were obtained and kept in the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, throughout the period of this experiment. They were housed in rat cages and were fed the standard rat diet. They were given access to clean water at all times and allowed to acclimatise for a period of 2 weeks. After acclimatisation, fasting blood glucose (pre-induction) was measured before induction of diabetes.

Antidiabetic study

Diabetes was induced in these rats by a single intraperitoneal (I.P.) injection of freshly prepared solution of alloxan monohydrate (100 mg/kg). Forty-eight hours after induction, fasting blood glucose level was assessed using ACCUCHEK active blood glucometer and a total of 40 animals with blood glucose >200 mg/dL were selected for the antidiabetic study.

A total of 50 rats were randomly allotted to five groups of 10 animals each. Group A animals were not diabetic and received vehicle + normal saline and served as control, Group B animals were diabetic rats and did not receive any treatment, Group C comprised diabetic rats that received glibenclamide at 4 mg/kg and Groups D and E received the MLVA at 200 mg/kg and 400 mg/kg, respectively. All treatments were done daily via the oral route and lasted for 28 days. Blood glucose level and weight of rats were measured weekly and blood was collected for haematology and serum for biochemical assays on days 14 and 28 post-treatment.

Measurement of fasting blood glucose and body weight

Fasting blood glucose was determined at intervals of 7 days during the 28-day experimental period using a glucometer (ACCUCHEK). Body weight of animals was also determined at intervals of 7 days using a weighing balance.

Blood collection, serum preparation and haematological analysis

Blood was collected for haematological evaluation on days 14 and 28 post-treatment. From each rat, 5 mL of fresh whole blood was collected through the retro-orbital venous plexus. Of the 5 mL of blood, 2 mL was used for haematological analysis. Haematological analysis was done for determining packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell (WBC) count, white blood cell differential count and platelets count. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using appropriate calculations. The remaining 3 mL of blood was also collected into sterile tubes and left for about 30 min to clot. The clotted blood was
thereafter centrifuged at 4000 rpm for 10 min. Serum was harvested into sample bottles and stored at −20°C until the time of analysis. The animals were not anaesthetised.

**Tissue preparation**

After collection of blood from each of the animals, all rats were sacrificed by cervical dislocation after the 28th day of the treatment and a portion of the intestine was harvested on ice, rinsed and homogenised in aqueous potassium buffer (0.1 M, pH 7.4) and the homogenate was centrifuged at 10 000 rpm (4°C) for 10 min to obtain the post-mitochondrial fraction (PMF).

**Biochemical assays**

**Serum chemistry profile**

A portion of each blood sample was collected (on days 14 and 28 post-treatment) into plain bottles and thereafter centrifuged to obtain serum which was used to estimate total cholesterol, triglycerides (TGs) and high-density lipoproteins (HDLs), using commercial kits (Randox Laboratories, Ltd. UK) and following standard procedures as outlined by the producer.

**Determination of intestinal glutathione peroxidase activity:**

The intestinal glutathione peroxidase (GPx) activity was also measured according to Beutler et al. (1963) The reaction mixture was subsequently incubated at room temperature for 30 min. The mixture was then mixed thoroughly by vortexing until it foamed and a light pink colour of the reaction mixture was observed. The mixture was thereafter read with a spectrophotometer at 412 nm using distilled water as blank.

**Determination of intestinal hydrogen peroxide generation:**

Hydrogen peroxide generation was determined according to Woff (1994). To 2.5 mL of 0.1 M potassium phosphate buffer (pH 7.4), 0.250 mL of ammonium ferrous sulphate (AFS), 0.1 mL of sorbitol, 0.1 mL of xylenol orange (XO), 0.025 mL of H$_2$SO$_4$ and 0.050 mL of intestinal PMF was added. The mixture was mixed thoroughly by vortexing until it foamed and a light pink colour of the reaction mixture was observed. The reaction mixture was subsequently incubated at room temperature for 30 min. The absorbance was assessed at 560 nm, using distilled water as blank. The H$_2$O$_2$ generated was extrapolated from the H$_2$O standard curve.

**Protein determination**: Protein concentrations were determined as described by Gornall et al. (1949); briefly, 1 mL of diluted serum was added to 3 mL of the Biuret reagent. The reaction mixture was incubated at room temperature for 30 min. The mixture was thereafter read with a spectrophotometer at 540 nm using distilled water as blank. The final value for total protein was extrapolated from the total protein standard curve.

**Histopathology**

Small pieces of pancreas were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5–6 µm in thickness were made and stained with haematoxylin and eosin for histopathological examination (Drury, Wallington & Cancerson 1976).

**Statistical analysis**

Results are expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA), using GraphPad Prism version 6. The level of statistical significance was considered as $p < 0.05$. Student’s $t$-test at 95% level of significance was used to assess significant difference between controls and the treated group.
TABLE 1: Effect of Vernonia amygdalina on blood glucose (mg/dL) of alloxan-induced diabetic rats.

| Groups | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|--------|-------|-------|--------|--------|--------|
| A      | 122.3 ± 7.70a,b  | 105.7 ± 8.65a,b  | 113.6 ± 13.45a  | 105.4 ± 6.39b  | 124.0 ± 5.28b  |
| B      | 489.0 ± 189.70a  | 427.5 ± 38.92a  | 481.0 ± 53.43a,b | 420.0 ± 59.40a  | 483.5 ± 70.04a  |
| C      | 600.0 ± 8.02a    | 406.0 ± 68.32a  | 419.33 ± 85.47a | 427.00 ± 27.58a | 442.20 ± 196.99a |
| D      | 333.4 ± 114.5a   | 118.2 ± 42.99a  | 163.6 ± 45.25a  | 163.6 ± 74.5a   | 108.4 ± 37.7b   |
| E      | 467.5 ± 84.6a    | 162.3 ± 52.32a  | 188.4 ± 89.4a   | 181.2 ± 84.4a   | 176.0 ± 15.3a   |

Values are mean ± SD, n = 10, p < 0.05 compared with control, p < 0.05 compared with diabetic control, p < 0.05 compared with glibenclamide, p < 0.05 compared with MLVA, 200 mg/kg and p < 0.05 compared with MLVA, 400 mg/kg.

TABLE 2: Effects of Vernonia amygdalina on body weight (g) of alloxan-induced diabetic rats.

| Groups | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|--------|-------|-------|--------|--------|--------|
| A      | 173.1 ± 28.9a  | 197.1 ± 29.2a  | 212.4 ± 30.1a  | 222.3 ± 45.7a,b | 233.6 ± 18.3a  |
| B      | 145.3 ± 7.4a   | 159.0 ± 3.1a   | 168.4 ± 4.7a   | 160.2 ± 13.8a   | 156.2 ± 13.8a   |
| C      | 160.0 ± 13.2a  | 140.0 ± 15.7a  | 131.4 ± 20.7a,b | 138.4 ± 24.1a   | 127.2 ± 19.3a   |
| D      | 186.0 ± 25.5a  | 170.0 ± 12.7a  | 166.8 ± 18.4a  | 203.7 ± 12.6a   | 200.4 ± 25.8a   |
| E      | 171.9 ± 25.5a  | 183.0 ± 33.7a  | 177.7 ± 41.3a  | 180.6 ± 38.7a   | 218.6 ± 25.9a   |

Values are mean ± SD, n = 10, p < 0.05 compared with control, p < 0.05 compared with diabetic control, p < 0.05 compared with glibenclamide, p < 0.05 compared with MLVA, 200 mg/kg and p < 0.05 compared with MLVA, 400 mg/kg.

TABLE 3: Effects of Vernonia amygdalina on packed cell volume, haemoglobin concentration and red blood cell count of alloxan-induced diabetic rats.

| Parameters | Groups | Basal | 14 days | 28 days |
|------------|--------|-------|---------|---------|
| PCV (%)    | A      | 47.00 ± 1.00 | 50.60 ± 3.97 | 44.20 ± 1.92 |
|            | B      | 43.40 ± 0.54 | 44.00 ± 2.00 | 43.50 ± 2.65a |
|            | C      | 44.20 ± 1.92 | 40.00 ± 1.00 | 46.75 ± 2.50 |
|            | D      | 43.40 ± 1.67 | 43.00 ± 1.41 | 47.40 ± 1.34a |
|            | E      | 47.00 ± 1.40 | 43.5 ± 0.59 | 48.20 ± 2.86 |
| HB (g/dL)  | A      | 15.54 ± 0.89 | 17.53 ± 0.42 | 16.22 ± 0.95 |
|            | B      | 14.23 ± 0.41 | 14.70 ± 0.08 | 14.00 ± 0.70a,b |
|            | C      | 14.64 ± 0.77 | 13.37 ± 0.51a | 15.80 ± 1.10a |
|            | D      | 14.83 ± 1.04 | 14.48 ± 0.40a | 15.88 ± 0.38 |
|            | E      | 15.73 ± 1.62 | 14.70 ± 0.08a | 16.24 ± 0.85 |
| RBC (×10^6/µL) | A      | 7.74 ± 0.32 | 8.83 ± 0.04 | 7.89 ± 0.72 |
|            | B      | 7.34 ± 0.34 | 7.47 ± 0.48 | 7.16 ± 0.30 |
|            | C      | 7.37 ± 0.38 | 6.87 ± 0.06b | 7.40 ± 0.15 |
|            | D      | 7.44 ± 0.51 | 7.49 ± 0.20a | 7.89 ± 0.41 |
|            | E      | 7.54 ± 0.47 | 7.65 ± 0.06a | 7.45 ± 0.29b |

Values are mean ± SD, n = 10, p < 0.05 compared with control, p < 0.05 compared with diabetic control, p < 0.05 compared with glibenclamide, p < 0.05 compared with MLVA, 200 mg/kg and p < 0.05 compared with MLVA, 400 mg/kg.

**Ethical considerations**

All experimental procedures were in conformity with the University of Ibadan Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal upkeep and use.

**Results**

**Effect of Vernonia amygdalina on blood glucose and body weight of alloxan-induced diabetic rats**

Alloxan monohydrate induces hyperglycaemia in rats. The MLVA caused a significant (p < 0.05) decrease in the fasting blood glucose of treated rats (Table 1) when compared with the diabetic control and the glibenclamide-treated group. This decrease was comparable with that of the normal control. Also, extract-treated groups showed statistically significant (p < 0.05) increases in weight gain at the end of 21 days when compared with diabetic control (Table 2).

**Haematological parameters**

The induction of diabetes caused statistically significant (p < 0.05) reductions in the PCV and Hb of diabetic rats compared with the normal control. However, treatment with MLVA resulted in a significant increase in the values of these parameters when compared with the diabetic untreated group. RBC values of extract-treated rats showed a non-significant increase when compared with the diabetic control group (Table 3).

Concerning haematometric indices, the following observations were noted: significant increase in the MCHC in rats treated with MLVA when compared with the diabetic control and glibenclamide-treated groups (Table 4). There were no significant differences in the values of MCV and MCH.

Platelet count of MLVA-treated diabetic rats was significantly (p < 0.05) decreased when compared with the diabetic control and glibenclamide-treated rats and was comparable with that of the normal control (Table 5). Total WBC and differentials were increased in diabetic rats when compared with normal control. Administration of MLVA resulted in a decrease in the total white blood cells (TWBC) and lymphocyte counts (Tables 6 and 7). The neutrophil count in untreated diabetic group showed significant increase when compared with the normal control and the diabetic groups treated with MLVA (Table 7).

Eosinophil count was significantly reduced in the untreated diabetic rats when compared with the normal control and the MLVA-treated groups. There were no significant changes in the monocyte counts (Table 7).

**Effect of Vernonia amygdalina on serum lipid profile of alloxan-induced diabetic rats**

From this study, treatment with *V. amygdalina* caused a statistically significant (p < 0.05) reduction in the levels of serum TGs and cholesterol (Table 8) in the diabetic rats. High-density lipoprotein levels showed significant increase (p < 0.05) in MLVA-treated groups when compared with the diabetic untreated group. However, there were no significant changes in the levels of low-density lipoproteins (LDLs) (Table 8).
**TABLE 4: Effects of Vernonia amygdalina on mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of alloxan-induced diabetic rats.**

| Parameters | Groups | Basal | 14 days | 28 days |
|------------|--------|-------|---------|---------|
| MCV (fl)   | A      | 59.24 ± 1.61 | 63.50 ± 3.58 | 60.04 ± 0.99 |
| B          | 58.31 ± 2.72 | 57.15 ± 2.36 | 58.05 ± 0.21 |
| C          | 60.08 ± 3.88 | 64.38 ± 2.25 | 58.45 ± 1.25 |
| D          | 60.34 ± 3.23 | 61.04 ± 3.47 | 58.13 ± 3.64 |
| E          | 61.13 ± 3.72 | 63.95 ± 3.70 | 56.67 ± 0.75 |
| MCH (pg)   | A      | 20.10 ± 0.99 | 20.06 ± 0.93 | 19.84 ± 0.51 |
| B          | 19.42 ± 0.76 | 19.75 ± 0.20 | 19.20 ± 0.53 |
| C          | 19.88 ± 0.46 | 20.46 ± 1.27 | 19.91 ± 0.70 |
| D          | 19.97 ± 0.85 | 20.25 ± 0.77 | 19.33 ± 0.22 |
| E          | 20.85 ± 1.48 | 21.55 ± 1.85 | 19.23 ± 0.15 |
| MCHC (g/dL)| A      | 33.73 ± 2.17 | 33.31 ± 0.63 | 33.55 ± 0.41 |
| B          | 32.82 ± 0.89 | 33.09 ± 1.67 | 32.56 ± 0.16** |
| C          | 33.41 ± 1.98 | 32.49 ± 2.78 | 33.41 ± 0.51** |
| D          | 32.48 ± 0.95 | 33.02 ± 0.92 | 34.71 ± 0.41** |
| E          | 32.71 ± 2.64 | 32.57 ± 0.82 | 34.00 ± 0.52** |

Values are mean ± SD, n = 10, *p < 0.05 compared with control, **p < 0.05 compared with alloxan.

**TABLE 5: Effects of Vernonia amygdalina on platelet count (10⁹/L) of alloxan-induced diabetic rats.**

| Parameters | Groups | Basal | 14 days | 28 days |
|------------|--------|-------|---------|---------|
| Lymphocyte | A      | 66.6 ± 6.2 | 79.00 ± 1.00 | 53.60 ± 1.94 |
| B          | 68.3 ± 5.6 | 71.75 ± 5.44 | 60.67 ± 6.66 |
| C          | 70.1 ± 3.8 | 60.33 ± 7.09** | 52.35 ± 5.68** |
| D          | 66.1 ± 3.9 | 71.17 ± 3.81 | 58.33 ± 1.15** |
| E          | 68.7 ± 5.0 | 62.00 ± 3.16** | 62.69 ± 0.58** |

**TABLE 7: Effects of Vernonia amygdalina on white blood cell differentials count of alloxan-induced diabetic rats.**

| Parameters | Groups | Basal | 14 days | 28 days |
|------------|--------|-------|---------|---------|
| Neutrophil | A      | 32.50 ± 0.58 | 13.67 ± 2.51 | 26.75 ± 2.22 |
| B          | 30.00 ± 3.81 | 29.67 ± 3.05** | 45.00 ± 3.61** |
| C          | 27.17 ± 3.06 | 24.00 ± 6.56 | 31.00 ± 5.20 |
| D          | 30.00 ± 3.16 | 22.00 ± 1.63** | 27.00 ± 3.56** |
| E          | 27.14 ± 4.63 | 34.00 ± 1.83** | 28.25 ± 3.40** |

**Histopathology of the pancreas**

Histopathological analysis of the pancreas was carried out in this study; diabetic control shows islets in varying sizes, few and far between, but the treated group show mild pathologies (Figure 1).

**Markers of oxidative stress**

The results obtained from this study revealed that induction of diabetes with alloxan monohydrate led to a significant (*p < 0.05*) increase in the H₂O₂ and MDA levels of the intestinal tissue (Figure 2). However, treatment with MLVA (200 mg/kg and 400 mg/kg) led to a significant (*p < 0.05*) reduction in the level of H₂O₂ generated and MDA content in the intestinal tissue (Figure 2).

**Effects of MLVA on protein thiols**

A significant (*p < 0.05*) reduction of thiol content was seen in diabetic untreated rats when compared with control, while treatment with MLVA at all doses (200 mg/kg and 400 mg/kg) led to a significant increase (*p < 0.05*) in the thiol content in intestinal homogenate of diabetic rats when compared with the untreated diabetic rats (Figure 3).

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Effects of MLVA on some anti-oxidant markers

A significant ($p < 0.05$) reduction in the activities of superoxide dismutase, glutathione peroxidase and glutathione-S-transferase (GST) was observed in the diabetic untreated rats when compared with normal control, while treatment with MLVA at all doses (200 mg/kg and 400 mg/kg) led to a significant increase ($p < 0.05$) in the activities of these enzymes in the intestinal homogenate of treated diabetic rats when compared with the untreated diabetic rats (Figure 4).

FIGURE 1: Photomicrograph of the pancreas of alloxan-induced diabetic rats (×400). (a) Normal control shows normal exocrine acini (blue arrows); the intralobular and interlobular ducts are essentially normal and contain pancreatic secretion (slender arrows). (b) Diabetic control shows islets in varying sizes, few and far between (slender arrows). (c) Glibenclamide-treated group shows islets in varying sizes, few and far between (blue arrows). (d) 200 mg/kg MLVA and (e) 400 mg/kg MLVA islets show hyalinisation in the centre while the remnant islet cells are limited to the periphery; these are few and far between (blue arrows).
FIGURE 2: Effect of methanol leaf extract of Vernonia amygdalina (MLVA) on (a) hydrogen peroxide levels and (b) malonaldehyde content in intestinal tissue of alloxan-induced diabetic rats. Values are mean ± SD, n = 5, *p < 0.05 compared with control, †p < 0.05 compared with diabetic control, ‡p < 0.05 compared with glibenclamide, §p < 0.05 compared with MLVA, 200 mg/kg and ‡p < 0.05 compared with MLVA, 400 mg/kg.

FIGURE 3: Effect of methanol leaf extract of Vernonia amygdalina (MLVA) on protein thiol (a), non-protein thiol (b) and reduced glutathione (GSH) (c) concentration in intestinal tissue of alloxan-induced diabetic rats. Values are mean ± SD, n = 5, *p < 0.05 compared with control, †p < 0.05 compared with diabetic control, ‡p < 0.05 compared with glibenclamide, §p < 0.05 compared with MLVA, 200 mg/kg and ‡p < 0.05 compared with MLVA, 400 mg/kg.
Diabetes mellitus, a global burden for a developing economy, is characterised by hyperglycaemia and results in disturbances in carbohydrate, fat and protein metabolism, which arise because of defects in insulin secretion or insulin action. It is presently a very prevalent disease, especially in Africa (Aguocha et al. 2013; Bos & Agyemang 2013). Uncontrolled hyperglycaemia can lead to increased production of free radicals, especially reactive oxygen species (ROS) and nitrogen oxygen species (NOS) (Robertson 2004).

In this study, Groups B, C, D and E induced with diabetes using alloxan developed hyperglycaemia. Alloxan is a diabetogenic agent with two distinct effects interfering with the physiological function of the pancreatic beta cells by selectively inhibiting glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it also causes a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells (Lenzen 2008).

In this study, blood glucose level of diabetic rats treated with 200 mg/kg and 400 mg/kg doses of the MLVA was reduced to that comparable with those of the normal control (Table 1). This is similar to other studies on the antihyperglycaemic effects of *V. amygdalina* (Atangwho et al. 2007; Ebong et al. 2008; Momoh, Akoro & Godonu 2014). This blood glucose lowering effect is the result of the bioactive secondary components of the plant. Relationships between hyperglycaemia and decrease in body weight of experimental animals have been reported (Igile et al. 1995; Zafar et al. 2009). In this study, there was a significant ($p < 0.05$) decrease in body weight of the diabetic untreated group compared with the normal control and extract-treated animals (Table 2). This is an indication of tissue wasting as a result of poor glycaemic control in diabetes mellitus and this usually results in an increase in protein and fat mobilisation, resulting in eventual weight loss (Atangwho et al. 2007; Cotran, Kumar & Collins 1999). The extract-treated group however showed significant ($p < 0.05$) increase in weight gain from day 21 of extract administration which was comparable with that of the normal control which may be the result of improved glycaemic control.

**Discussion**

Diabetes mellitus, a global burden for a developing economy, is characterized by hyperglycaemia and results in disturbances in carbohydrate, fat and protein metabolism, which arise because of defects in insulin secretion or insulin action. It is presently a very prevalent disease, especially in Africa (Aguocha et al. 2013; Bos & Agyemang 2013). Uncontrolled hyperglycaemia can lead to increased production of free radicals, especially reactive oxygen species (ROS) and nitrogen oxygen species (NOS) (Robertson 2004).

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A wide range of haematology laboratory values change significantly in patients with diabetes. Anaemia has been reported as a complication of diabetes mellitus (Kothari & Bokariya 2012). It is the result of an increase in non-enzymatic glycosylation of RBC membrane proteins. Oxidation of these proteins in the presence of hyperglycaemia as seen in diabetes mellitus results in lyses of the blood cells and so anaemia ensues (Oyedemi, Yakubu & Afolayan 2011). Another dominant factor that contributes to the increased prevalence of anaemia in diabetes is the failure of the kidney to increase erythropoietin production in response to falling haemoglobin. In this study, we observed that at 28 days following induction of diabetes, the values of PCV, Hb and MCHC, as indices of anaemia, were significantly lower among diabetic untreated rats when compared with normal controls. This agrees with a previous study that observed that the mean values of Hb, PCV and MCHC for the diabetic patients are lower than the values of the control group, indicating the presence of anaemia in the former group (Kothari & Bokariya 2012). However, treatment with MLVA resulted in a significant increase in the values of these parameters in the treated diabetic group. This gives an indication that V. amugdalina contains bioactive principles that can stimulate the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce RBCs (Ohlsson & Aber 2012). The stimulation of this hormone enhances synthesis of RBC, which is supported by the improved level of MCH and MCHC (Abu-Zaiton 2010). These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen-carrying capacity of the blood.

Platelets of diabetic patients are characterised by dysregulation of several signalling pathways and have been suggested to be hyperreactive, showing increased adhesion, activation and aggregation (Randriamboavonjy & Fleming 2012). The glycation of platelet surface proteins reduces membrane fluidity and increases platelet adhesion, causing incorporation of glycated proteins into the thrombi. An increase in calcium mobilisation from intracellular storage pools, resulting in increased intracellular calcium levels, has also been correlated with reduction in membrane fluidity (Jokl 1997). Insulin has a direct inhibitory effect on platelet aggregation. Insulin binds to platelet membrane receptors and reduces platelet response to thrombin, ADP, arachidonic acid, collagen and platelet-activating factor. Diabetic platelets are less sensitive to this inhibitory action of insulin (Falcon et al. 1988) which suggests that reduced insulin sensitivity may account for platelet hyperreactivity in this condition (Hunter & Hers 2009). Clinically elevated platelet counts are frequently seen in diabetics with a long duration of disease. Elevated platelet levels as well as platelet dysfunction could be injurious to microcirculation and enhance the risk for vascular complications. A previous report seems to suggest the possibility that elevated platelet count could be used as a prognostic indicator for future diabetic complications (Sterner, Carlson & Ekberg 1998). In this study, elevated platelet counts were observed in diabetic rats compared with normal control. These elevations were reduced following treatment with MLVA at all doses tested in a dose-dependent manner. This could be the result of the ability of the extract to increase the sensitivity of the platelets to insulin, thereby causing a reduction in platelet aggregation.

Peripheral blood leukocytes are composed of polymorphonuclear cells, including monocytes as well as lymphocytes. Polymorphonuclear and mononuclear leukocytes can be activated by advanced glycation end products (Pertynska-Marczewska et al. 2004), oxidative stress (Shurtz-Swirski et al. 2004), angiotensin II (Lee et al. 2004) and cytokines (Scherberich 2003) in a state of hyperglycaemia. Changes in TWBC have also been associated with insulin resistance and complications of cardiovascular disease (CVD) (Mohammed et al. 2013; Uko et al. 2013). The result of this study showed a significant increase in TWBC, lymphocytes and neutrophils of diabetic rats when compared with normal control. After the administration of MLVA to diabetic rats, however, a non-significant decrease in the leucocytes count was observed which could be the result of the anti-inflammatory property of the plant which leads to amelioration of the low-grade inflammatory state seen in diabetes as a result of insulin resistance (Chen et al. 2006; Vozarova et al. 2002). This agrees with other reports (Edet, Patrick & Olorunfemi 2013; Tanko et al. 2008) implying a reduction in the process of advance glycation and oxidative stress within the blood cells.

The pathogenesis of diabetes mellitus is associated with disturbances in carbohydrate, fat and protein metabolism. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses TGs. However, in diabetic state, lipoprotein lipase is not activated because of insulin deficiency, resulting in hyper triglyceridaemia (Cianflone, Paglialunga & Roy 2008) and hyper cholesterol dementia owing to metabolic abnormalities. The dyslipidaemia is characterised by increase in total cholesterol (TC), LDLs, very low-density lipoproteins (VLDLs), TGs and a fall in HDL. In this study, the levels of TC and total TG were markedly increased following induction of diabetes with alloxan confirming that the dyslipidaemia associated with diabetes mellitus and these alterations in the levels of major lipids such as cholesterol and TGs can give useful information on lipid metabolism as well as indicate predisposition of the animals to cardiovascular risk (Yakubu, Akanji & Oladiji 2008). The altered serum lipid profile was, however, reversed significantly (p < 0.05) following treatment with the MLVA. This is in agreement with the study by Iwara et al. (2015).

Oxidative stress has recently been recognised as a significant player in the pathogenesis of gastrointestinal complications of diabetes (Kashyap & Farrugia 2011). Hyperglycaemia generates ROS, which in turn causes damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in diabetes mellitus (Hunt, Dean & Wolff 1988; Jaganjac et al. 2013). Research data indicate that in diabetic patients, the gastric cells are subject to oxidative...
stress (Lomax, Sharkey & Furness 2010). Excessive production of glucose-derived free radicals in diabetes then causes damage to cellular proteins, lipids and eventually cell death as free radicals react with lipids causing peroxidation, leading to the release of products such as malondialdehyde (MDA), hydrogen peroxide and hydroxyl radicals (Pompeilla et al. 1991), resulting in damages to the gastric mucosa (Chandrasekharan et al. 2011).

The results of this study showed that there is oxidative damage in the intestine of diabetic rats evidenced by the increase in H₂O₂ levels and MDA content of diabetic control rats when compared with the normal control. Furthermore, significant decrease in reduced glutathione, protein thiol (PT) and non-protein thiol (NPT) levels in diabetic control rats when compared with the normal control was observed. Also, the activities of the antioxidant enzyme systems (SOD, GPx and GST) were significantly (p < 0.05) reduced in the diabetic control rats. The observed decrease in SOD activity could result from inactivation by H₂O₂ or by glycation of the enzyme, which has been reported to occur in diabetes (Sozmen et al. 2001) as a result of depletion owing to excessive use of these enzymes to mop up the hyperglycaemia-induced free radical generation. Also, these enzymes are targets of glycation which can lead to inhibition of their enzymatic activity (Jung 2003). GPx and GST work together with glutathione in the decomposition of H₂O₂ and other organic hydroperoxides to non-toxic products at the expense of the GSH (Bruce, Freeman & James 1982). The decreased activity of GST observed in diabetic state may be because of the inactivation caused by ROS (Andallu & Varadacharyulu 2003).

Treatment with MLVA resulted in a decrease in the levels of H₂O₂ and MDA compared with the diabetic control rats. A number of earlier investigators have shown that the antioxidant effect of plant products is mainly because of radicals scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins and phenolic terpenes (Hu et al. 2010). These phytochemicals were found to be essential components of V. amygdalina (Adetutu, Oyewo & Adesokan 2013). The reduced MDA may therefore be related to the antioxidant properties of the phytochemical compounds found in the extract, as compounds, such as flavonoids and tannins, have been reported to exert antioxidant activity by scavenging free radicals that cause H₂O₂ lipid peroxidation (Usunobun & Okolie 2015; Usunobun et al. 2015; Zhao et al. 2007). GSH, the thiol group, has been shown to have antioxidant power and reduction in the thiol content has been implicated in oxidative stress (Li et al. 2015).

In this study, MLVA administration resulted in a significant (p < 0.05) increase in reduced glutathione (GSH), NPT and PT levels in treated diabetic rats when compared with the diabetic control.

Oxidative stress acts on signal transduction and, via NF-κB, affects gene expression of antioxidant enzymes, thereby reducing the expression of antioxidant enzymes. Also, hyperglycaemia can simply inactivate existing enzymes by glycatating these proteins (Wienspersger 2003). The significant increase in the activity of antioxidant enzymes SOD, GPx and GST observed in the intestinal homogenates of MLVAtreated rats in this study could be the result of the ability of MLVA to prevent the inactivation of the antioxidants or increase their expression by downregulating the expression of NF-κB.

This study concludes that V. amygdalina possesses antidiabetic properties and holds great promise in the development of new therapies for ameliorating hyperglycaemia-induced oxidative stress in diabetics. The study thus showed that this plant can prevent or protect diabetics from complications, thereby improving their quality of life.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors’ contributions

A.A.A. was the project leader and was responsible for experimental and project design. A.T.A., A.A.O., T.O.O. and A.D.A. performed most of the experiments. A.E.A. made conceptual contributions and plant identification. All authors read and approved the final draft of the manuscript.

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