

Full Length Research Paper

Effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* extract on the activity of angiotensin converting enzyme, hypolipidemic and antioxidant parameters in streptozotocin-induced diabetic rats

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This study was aimed at investigating the angiotensin converting enzyme (ACE) inhibitory effect, hypolipidemic and antioxidant properties of combined extract of *Ocimum gratissimum* and *Vernonia amygdalina* in streptozotocin-induced diabetic rats. Thirty (30) apparently healthy male Wistar rats were grouped into: control, diabetic, diabetic + insulin, diabetic + 100 mg/kg and diabetic + 250 mg/kg extract groups. All rats except those in the control group were made diabetic using a single intraperitoneal injection of 60 mg/kg streptozotocin. Administration of extract began four days after induction of diabetes and lasted for four weeks. Results show a significant ($P<0.05$) increase in serum and liver triacylglycerol, total cholesterol and low density lipoprotein levels upon induction of diabetes, which reduced significantly ($P<0.05$) in rats given insulin and the extract. HDL-C levels remained unchanged in both liver and serum of diabetic rats when compared to control rats. The serum levels of sulfuroxide dismutase (SOD) and catalase increased significantly ($P<0.05$) in rats given insulin and the extract, while thiobarbituric acid reactive substances decreased significantly ($P<0.05$) compared to diabetic rats. The angiotensin converting enzyme (ACE) activity in lungs, kidney and heart of diabetic rats were significantly ($P<0.05$) higher than that of control rats. Also kidney and liver functions were affected on induction of diabetes with a significant increase in levels of urea and aspartate aminotransferase (AST). All of the above factors reduced significantly ($P<0.05$) when rats were given either insulin or the extract. In conclusion, this study has shown that a combination of the aqueous leaf extracts of *Vernonia amygdalina* and *Ocimum gratissimum* inhibits ACE activity, lowers blood glucose and lipid levels; and is a good antioxidant.

Key words: Angiotensin converting enzyme (ACE), *Ocimum gratissimum* and *Vernonia amygdalina*, diabetes.

INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality (Rang et al., 1991). According to the World health organization (WHO), there are over 150

million diabetics worldwide and this is likely to increase to about 300 million by the year 2023, in spite of major inroads in understanding the pathophysiology and treatment of the disease (WHO, 1999). Experimental diabetes

in animals has provided a considerable insight into the physiologic and biochemical derangements of the diabetic state (Magarinos and McEwen, 2000; Srinivasan and Ramarao, 2007).

Studies have shown that individuals with diabetes have a higher incidence of liver and kidney function abnormalities, as well as formation of free radicals due to glucose oxidation, non-enzymatic glycosylation of proteins and subsequent oxidative degradation of glycated proteins, leading to a decline in antioxidant defence mechanisms and damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance (Kangralkar et al., 2010; Arora, 2010).

Diabetes mellitus is a complex metabolic disease caused by impairment of insulin signaling path ways and usually results from pancreatic β -cells deficiency and/ or a deficiency of insulin. It is associated with profound alteration in serum lipid and lipoprotein profile with an increase risk in coronary heart disease (Betteridge, 1997; Arora, 2010).

Studies have also demonstrated the possible role of renin angiotensin aldosterone system (RAAS) in the pathogenesis of glomerular injury in diabetes mellitus, with conflicting results on activity of ACE, the major enzyme regulating angiotensin II production (Scherthaner et al., 1984). Also, the ability of ACE inhibitors and some direct renin inhibitors to prevent or show the progression of some complications of diabetes further suggest a pathologic role for RAAS in diabetes mellitus (Scherthaner et al., 1984).

Diabetes without proper treatments can lead to complications such as hypoglycaemia, diabetic ketoacidosis or non-ketotic hyperosmolar coma. More serious complications associated with long term diabetes include cardiovascular diseases, chronic renal failure, renal damage and neuropathy with damage to extremities. Adequate treatment is therefore important. However, population increase, inadequate drug supply, exorbitant cost of treatment and side-effects of several conventional drugs have increased the dependence on plant materials as source of medicine for a variety of ailments, many of which are yet to be scientifically validated (Yaro et al., 2007).

Ocimum gratissimum (basil) and *Vernonia amygdalina* (commonly known as bitter leaf) have been reported to have antidiabetic properties (Mohammed et al., 2007). However, very little have been documented on the combined use of both plants with respect to diabetes. Therefore, this work was designed to investigate the effects of the combined use of both plants on blood glucose, activity of ACE and some bio-chemical parameters in an animal model of diabetes.

MATERIALS AND METHODS

Plant material

The leaves of *O. gratissimum* and *V. amygdalina* were obtained

from Ibadan, Oyo state, Nigeria, in the month of March, 2010. They were authenticated by the Botanist at the department of Biological sciences, Faculty of Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Preparation of extract

The fresh leaves were air dried and made into powder by grinding. 100 g of the powdered leaves was soaked in 500 ml of distilled water for 48 h. The extract was sieved using a muslin cloth and then filtered under suction pressure with a Whatman's filter paper.

All extracts were then concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland), lyophilized (Christ Alpha 1-2 LD, Germany) and stored at 4°C until needed.

Animals

Thirty (30) apparently healthy male Wistar rats (*Rattus norvegicus*) weighing between 150 to 250 g were obtained from Department of Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. The animals were allowed to acclimatize for two weeks before commencement of experiment. They were allowed free access to water and feed (Vital Agricultural feeds, Zaria, Kaduna State) throughout the period of the experiment. All experimental protocol were assessed and approved by the Animal Care and Use Committee of Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Experimental design

Diabetes was induced by single intraperitoneal dose of 60 mg/kg of streptozotocin (STZ) dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 into 12 h-fasted rats. On the third day of STZ-injection, the rats were fasted for 6 h and blood glucose determined (Elias et al., 1994). Rats given the extracts and distilled water were treated by oral intubation for four weeks after induction of diabetes.

The rats were divided into five groups of six rats each:

Group 1: Control group; rats were normoglycemic and given distilled water

Group 2: Diabetic group; rats were diabetic, but not treated.

Group 3: Diabetic + insulin; rats were diabetic and treated with insulin (5 iu/100 g).

Group 4: Diabetic + 100 mg/kg extract.

Group 5: Diabetic + 250 mg/kg extract.

Acute toxicity studies

Acute oral toxicity studies of the combined extracts of *O. gratissimum* and *V. amygdalina* were carried out as described by Lorke (1983) with oral administration of increasing doses of the extracts from 10 mg/kg to 5000 mg/kg, while animals were observed for behavioural changes, toxicity and mortality for 24 h.

Determination of fasting blood glucose

Blood samples were collected from the tail of the rats and blood glucose levels determined by the glucose oxidase method using the ONE TOUCH Basic. The results were expressed as mg/dl (Rheney and Kirk, 2000).

Sample collection

At the end of the experiment, rats were sacrificed under anaesthesia and blood collected by cardiac puncture. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) bottles and centrifuged to obtain serum. Lungs, kidneys and hearts were rapidly removed, cleaned of fatty and connective tissues, blotted dry, weighed and stored at 4°C until use.

Determination of antioxidant activity of the extracts

Thiobarbituric acid reactive substances were determined as described by Buege and Aust (1978). Superoxide dismutase activity was determined using the McCord and Fridovich method (1988) and catalase was measured as described by Machly and Chance (1959).

Determination of ACE Activity

The ACE activity was determined as described by Cushman and Cheung (1971). Organs were homogenised in cold Trizma-HCl buffer (pH = 7.8) (Sigma) and centrifuged at 4°C for 15 min and 5000 x g. The supernatants were used for the assay. Briefly, the sample (0.2 mL) was added to ACE solution (50 µL) and the reaction started by adding 0.2 mL of 5 mmol/L hippuric histidyl leucine. This was incubated at 37°C for 15 min. The reaction was terminated by adding 0.25 mL of 1.0 N hydrochloric acid and then 2.0 mL ethyl acetate to extract the hippuric acid formed by the action of ACE. This was centrifuged at 3600 x g for 2 min, and 1 mL of upper layer transferred into a microcentrifuge tube and heated by dry bath at 100°C for 15 min to remove ethylacetate by evaporation. The resulting hippuric acid was dissolved in 3.0 mL of distilled water, and the absorbance read at 228 nm using a spectrophotometer (Jenway 6100, Dunmow, Essex, U.K.). The activity was determined by:

$$\text{Units/ml enzyme} = \frac{(\text{A}_{228\text{nm}} \text{ Test} - \text{A}_{228\text{nm}} \text{ Blank})}{(2)(3)(a)(b)(c)(d)}$$

Where; 2 = Conversion factor since the hippuric acid detected was 1/2 of the total amount produced in the assay; 2 mL of ethyl acetate was added and 1 mL of the organic layer containing the product, hippuric acid, was removed; 3 = total volume of hippuric acid solution; a = millimolar extinction coefficient of hippuric acid at 228 nm; b = time (in minutes) of the assay as per the unit definition; c = extraction efficiency of ethyl acetate, d = volume (in milliliter) of enzyme used.

Activity in tissues was expressed in units which corresponded to 1 µmol of hippuric acid released by enzymatic hydrolysis of HHL per minute per milligram of tissue.

Liver lipid extraction

Liver lipids were extracted as described by Folch et al. (1957). Briefly, 1 g of each tissue sample was homogenized in 20 ml chloroform/methanol, agitated for 15 to 20 min in an orbital shaker and centrifuged (3500xg) to recover the liquid phase. Water (0.2 ml) was added to the recovered solvent vortexed at 2000 rpm for some seconds and centrifuged at 3500 xg to separate the two phases. The lower phase containing liver lipids was collected for lipid profile determination.

Determination of biochemical parameters

The biochemical analysis of serum and liver samples was performed using reagents kits (Randox Kits). Biochemical parameters measured were aspartate aminotransferase, alanine aminotransferase, creatinine, cholesterol, triacylglycerol, total cholesterol, HDL-cholesterol and LDL-cholesterol.

Statistical analysis

The results were analyzed for statistical significance by one way analysis of variance (ANOVA) using the SPSS 17 statistical program and Post Hoc Test (LSD) between groups. All data were expressed as mean ± SD. P values < 0.05 were considered significant.

RESULTS

Acute toxicity

In the acute toxicity study, a single oral administration of combined extract of *Ocimum gratissimum* and *Vernonia amygdalina* at doses from 10 to 5000 mg/kg did not produce any apparent toxic symptom or mortality after the 24 h observation period.

Effect of *O. gratissimum* and *V. amygdalina* on blood glucose of streptozotocin-induced diabetic rats

The antidiabetic effect of the extract of *O. gratissimum* and *V. amygdalina* on fasting blood sugar levels of diabetic rats is shown on Table 1. The administration of streptozotocin (60 mg/kg) led to over 100% increase in blood glucose levels when compared to control rats (130.16 ± 17.16 mg/kg). Four weeks after daily treatment with insulin and the extract at 100 and 250 mg/kg, fasting blood glucose levels significantly ($P < 0.05$) reduced in diabetic rats. The glucose level in diabetic untreated rats increased from 130.16 ± 17.16 to 178.33 ± 27.55 mg/dl, while that of rats in the control group did not increase significantly. Treatment of rats with insulin significantly ($P < 0.05$) decreased blood glucose levels by 52% (from 140.00 ± 10.95 to 67.21 ± 8.75 mg/dl); the 100 mg/kg extract decreased glucose level by 21.93% (from 114.00 ± 10.67 to 89.33 ± 7.71 mg/dl) and the 250 mg/kg extract decreased blood glucose by 47.23% (from 148.50 ± 24.59 to 78.83 ± 9.75 mg/dl).

Effect of *O. gratissimum* and *V. amygdalina* on serum and liver lipid profile of streptozotocin-induced diabetic rats

The effect of the extract on diabetes-induced hyperlipidemia was also studied. It was observed that induction of diabetes increased triacylglycerols (TAG) from 169.89 ± 6.08 to 262.9 ± 36.63 mg/dl, TC (259.00 ± 8.11 mg/dl) and LDL-C (from 223.66 ± 46.50 to 383.24 ± 39.59 mg/dl)

Table 1. Fasting blood glucose levels of rats after before and after four weeks treatment.

FBG	Control	Diabetic	Diabetic + Insulin	Diabetic + 100 mg/kg extract	Diabetic + 250 mg/kg extract
Initial (mg/dl)	63.00 ± 9.95 ^a	130.16 ± 17.16 ^b	140.00 ± 10.95 ^a	114.00 ± 10.67 ^c	148.50 ± 24.59 ^e
Final (mg/dl)	70.20 ± 6.87 ^a	178.33 ± 27.55 ^c	67.21 ± 8.75 ^u	89.33 ± 7.71 ^u	78.83 ± 9.75 ^l

a, b, c, d, e, f Mean values within the same column with different superscript letters are significantly different ($P < 0.05$)

Table 2. Effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* on serum lipid profile of streptozotocin-induced diabetic rats.

Parameter (mg/dl)	Control	Diabetic	Diabetic + insulin	Diabetic + 100 mg/kg extract	Diabetic + 250 mg/kg extract
TAG	169.89 ± 6.08 ^a	262.9 ± 36.63 ^b	169.98 ± 12.16 ^a	201.03 ± 64.76 ^{a,b}	165.58 ± 24.80 ^a
TC	196.70 ± 25.78 ^a	259.00 ± 8.11 ^b	184.26 ± 3.17 ^a	185.39 ± 52.36 ^a	134.38 ± 48.23 ^a
HDL-C	23.09 ± 10.79 ^a	18.87 ± 3.94 ^a	30.36 ± 10.85 ^a	20.72 ± 6.02 ^a	27.96 ± 18.26 ^a
LDL-C	223.66 ± 46.50 ^a	383.24 ± 39.59 ^u	240.52 ± 14.38 ^a	248.79 ± 84.60 ^a	195.48 ± 47.43 ^a

Values are mean ± SD. ^{a, b} Mean values with different superscript along the same row are significantly different ($P < 0.05$). TAG, Triacylglycerol; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

Table 3. Effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* on liver lipid profile of streptozotocin-induced diabetic rats.

Parameter (mg/dl)	Control	Diabetic	Diabetic + insulin	Diabetic + 100 mg/kg extract	Diabetic + 250 mg/kg extract
TAG	216.21 ± 45.88 ^{a,c}	251.98 ± 52.76 ^a	127.97 ± 35.55 ^b	158.72 ± 29.76 ^{b,c}	209.51 ± 58.70 ^{a,b}
TC	177.78 ± 29.92 ^a	359.26 ± 24.32 ^b	161.11 ± 41.07 ^a	169.45 ± 33.17 ^a	166.73 ± 45.05 ^a
HDL-C	46.00 ± 9.09 ^{ab}	55.01 ± 12.60 ^{ab}	36.37 ± 12.17 ^{ab}	38.62 ± 8.55 ^{ab}	37.86 ± 15.39 ^{ab}
LDL-C	207.00 ± 76.60 ^a	418.79 ± 53.66 ^u	183.13 ± 39.12 ^a	174.48 ± 72.83 ^a	207.11 ± 65.90 ^a

Values are mean ± SD. ^{a, b} Mean values with different superscript along the same row are significantly different ($P < 0.05$). TAG, Triacylglycerol; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

levels significantly ($P < 0.05$) compared to rats in the control group. The levels of HDL-C in diabetic rats (18.87 ± 3.94 mg/dl), though lower than control rats (23.09 ± 10.79 mg/dl) was not significantly ($P > 0.05$) different. Treatment of diabetic rats with insulin significantly ($P < 0.05$) reduced TAG (169.98 ± 12.16 mg/dl), LDL-C (240.52 ± 14.38 mg/dl), and TC (184.26 ± 3.17 mg/dl). The reduction of TAG by the extract was dose-dependent, with rats given 250 mg/kg of the extract having significantly lower TAG (165.58 ± 24.80 mg/dl) than those given 100 mg/kg (201.03 ± 64.76 mg/kg). Also, LDL-C and TC decreased significantly in rats treated with both doses of the extract. There was no significant difference ($P > 0.05$) in HDL-C levels of diabetic rats and rats given insulin and the extract at both doses (Table 2).

The effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* on liver lipid profile of strep-

tozotocin-induced diabetic rats is shown on Table 3. From the results, the levels of TC (359.26 ± 24.32 mg/dl) and LDL-C (418.79 ± 53.66 mg/dl) increased significantly ($P < 0.05$) in diabetic rats compared to rats in the control group. Treatment of diabetic rats with insulin, 100 and 250 mg/kg of the extract significantly decreased TC (161.11 ± 41.07 , 169.45 ± 33.17 and 166.73 ± 45.05 mg/dl) to levels not significantly ($P > 0.05$) different from rats in the control group (177.78 ± 29.92 mg/dl). The TAG (251.98 ± 52.76 mg/dl) level of diabetic rats was not significantly ($P < 0.05$) different from control rats, but it reduced significantly ($P < 0.05$) when rats were given insulin (127.97 ± 35.55 mg/dl) and the extract at 250 mg/kg (158.72 ± 29.76 mg/dl). However, there was no significant ($P > 0.05$) difference in HDL level in untreated diabetic rats (55.01 ± 12.60 mg/dl) and those given insulin (36.37 ± 12.17 mg/dl), 100 mg/kg (38.62 ± 8.55 mg/dl)

Table 4. Effect of *Ocimum gratissimum* and *Vernonia amygdalina* on some oxidative stress parameters of streptozotocin-induced diabetic rats.

Paramater	Control	Diabetic	Diabetic + insulin	Diabetic + 100 mg/kg extract	Diabetic + 250 mg/kg extract
TBARS (nmol/mg protein)	0.672 ± 0.24 ^a	2.04 ± 1.21 ^b	0.804 ± 0.25 ^a	0.731 ± 0.01 ^a	0.787 ± 0.23 ^a
SOD (u/ml)	88.23 ± 1.12 ^a	33.92 ± 0.26 ^b	57.02 ± 1.03 ^c	58.52 ± 2.12 ^c	68.02 ± 0.60 ^d
Catalase (μmol/min)	68.35 ± 0.45 ^a	22.92 ± 3.02 ^u	56.30 ± 2.73 ^c	53.50 ± 1.03 ^c	66.25 ± 1.85 ^a

Values are mean ± SD. ^{a, b, c, d} Mean values with different superscript along the same row are significantly different ($P < 0.05$). TBARS, Thiobarbituric acid reactive substances; SOD, superoxide dismutase.

Table 5. Effect of *Ocimum gratissimum* and *Vernonia amygdalina* on some liver and kidney function parameters of streptozotocin-induced diabetic rats.

Paramater	Control	Diabetic	Diabetic ± insulin	Diabetic ± 100 mg/kg extract	Diabetic ± 250 mg/kg extract
AST (IU/L)	12.49 ± 3.91 ^a	22.16 ± 2.57 ^b	12.00 ± 1.96 ^a	12.90 ± 1.93 ^a	12.30 ± 1.70 ^a
ALT (IU/L)	52.50 ± 10.08 ^a	63.90 ± 15.50 ^a	51.00 ± 0.08 ^a	57.55 ± 2.64 ^a	52.45 ± 7.71 ^a
Creatinine (mg/dl)	13.00 ± 3.83 ^{a,b}	16.26 ± 2.23 ^b	12.33 ± 1.95 ^{a,b}	11.32 ± 2.75 ^a	10.22 ± 0.70 ^a
Urea (mg/dl)	28.57 ± 7.15 ^a	45.23 ± 10.91 ^u	30.95 ± 4.12 ^a	30.95 ± 3.35 ^a	28.90 ± 2.72 ^a

Values are mean ± SD. ^{a, b} Mean values with different superscript along the same row are significantly different ($P < 0.05$). AST, Alanine aminotransferase; ALT, aspartate aminotransferase.

and 250 mg/kg (37.86 ± 15.39 mg/dl).

Effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* on some oxidative stress parameters of streptozotocin-induced diabetic rats

The induction of diabetes significantly ($P < 0.05$) increased TBARS from 0.672 ± 0.24 nmol/mg protein in control rats to 2.040 ± 1.21 nmol/mg protein in diabetic rats. Administration of insulin, 100 and 250 mg/kg of the extract significantly ($P < 0.05$) decreased TBARS in serum of diabetic rats. There was no significant difference ($P > 0.05$) in TBARS levels of rats in the control group (0.672

± 0.24 nmol/mg protein), rats treated with insulin (0.804 ± 0.25 nmol/mg protein), 100 (0.731 ± 0.01 nmol/mg protein) and 250 mg/kg (0.782 ± 0.23 nmol/mg protein) of the extract.

The activity of SOD in rats within the control group (88.23 ± 1.12 U/ml) was significantly higher ($P < 0.05$) than diabetic rats (33.92 ± 0.26 U/ml). The SOD activity increased significantly when rats were given insulin (57.02 ± 1.03 U/ml) and the extract at 100 mg/kg (58.52 ± 2.12 U/ml) and 250 mg/kg (68.02 ± 0.60 U/ml). The effect of the extract on SOD was dose-dependent as serum SOD activity of rats given 250 mg/kg extract was significantly higher than that of rats given 100 mg/kg.

Diabetic rats had significantly lower ($P < 0.05$) serum catalase activity (22.92 ± 3.02 μmol/min) compared to rats in the control group (68.35 ± 0.45 μmol/min). There

was no significant ($P > 0.05$) difference in serum catalase activity of diabetic rats given insulin (56.30 ± 2.73 μmol/min) and 100 mg/kg of the extract (53.50 ± 1.03 μmol/min), although both groups had significantly ($P < 0.05$) higher serum catalase activity when compared to diabetic rats. The serum catalase activity of rats given 250 mg/kg extract (66.25 ± 1.85 μmol/min) was significantly ($P < 0.05$) higher than that of diabetic rats given the extract at 100 mg/kg (53.50 ± 1.03 μmol/min), showing a dose-dependent effect (Table 4).

Effect of *O. gratissimum* and *V. amygdalina* on some liver and kidney parameters of streptozotocin-induced diabetic rats

From the Table 5, diabetes significantly ($P < 0.05$) increased the serum levels of AST (22.16 ± 2.57 U/L) and urea (45.23 ± 10.91 U/L) than rats in the control group. This decreased significantly in rats given insulin and both doses of the extract. The serum AST levels of rats treated with insulin (12.00 ± 1.96 U/L), the extract at 100 mg/kg (12.90 ± 1.93 U/L) and 250 mg/kg (12.30 ± 1.70 U/L) were not significantly different from each other but were significantly ($P < 0.05$) lower than AST levels in serum of untreated diabetic rats. There was no significant ($P > 0.05$) difference in serum ALT of untreated diabetic rats (63.90 ± 15.50 U/L), rats treated with insulin (51.00 ± 0.08 U/L), those given the extract at 100 mg/kg (57.55 ± 2.64 U/L) and 250 mg/kg (52.45 ± 7.71 U/L) when

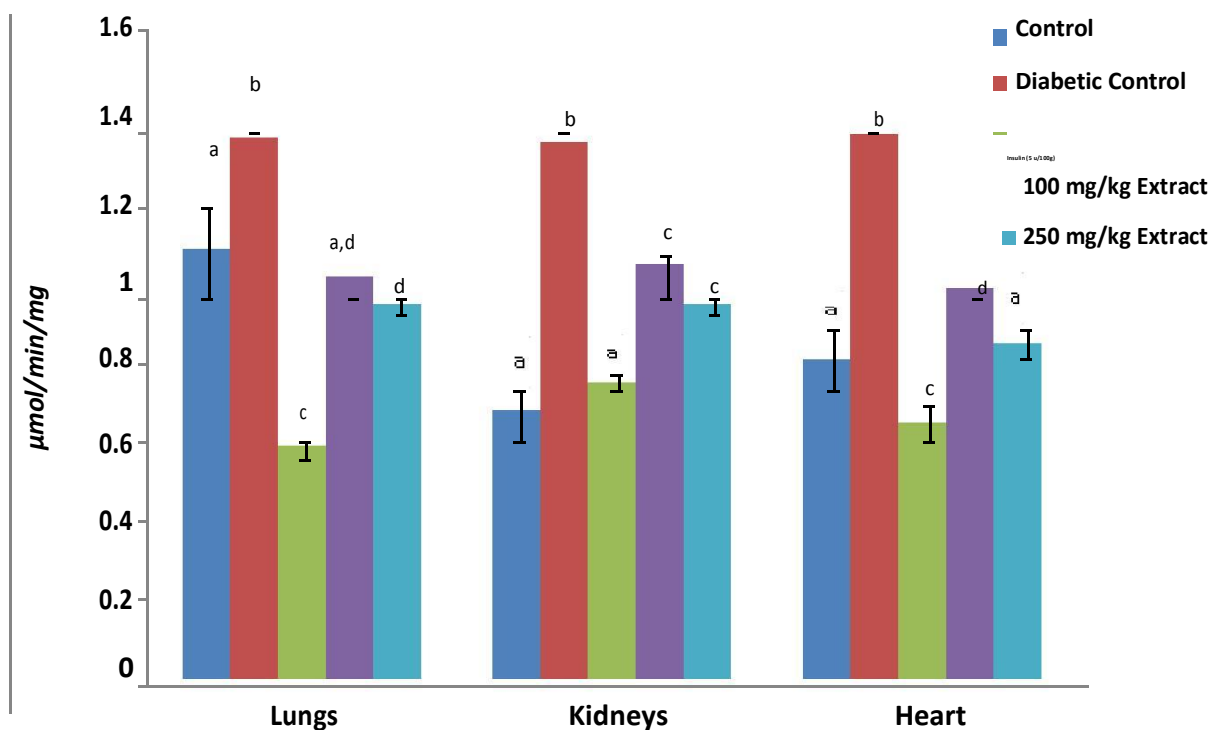


Figure 1. Effect of *Ocimum gratissimum* and *Vernonia amygdalina* on the activity of angiotensin converting enzyme in streptozotocin-induced diabetic rats. ^{a, b, c, d} Mean values within the same group having different superscript letters are significantly different ($P < 0.05$).

compared to control rats (52.50 ± 10.08 U/L).

The serum creatinine level of untreated diabetic rats (16.26 ± 2.23 mg/dl) was not significantly ($P < 0.05$) different from control rats (13.00 ± 3.83 mg/dl) and rats given insulin. However, it decreased significantly ($P < 0.05$) when rats were given the extract at 100 mg/kg (11.32 ± 2.75) and 250 mg/kg of the extract (10.22 ± 0.70 mg/dl) compared to the untreated diabetic rats. Also, serum urea levels decreased significantly ($P < 0.05$) in diabetic rats given insulin (30.95 ± 4.12 mg/dl), the extract at 100 mg/kg (30.95 ± 3.35 mg/dl) and 250 mg/kg (28.90 ± 2.72 mg/dl) when compared to untreated diabetic rats (45.23 ± 10.91 mg/dl).

Effect of combined extract of *V. amygdalina* and *O. gratissimum* on tissue ACE activity of streptozotocin-induced diabetic rats

The effect of the extract on tissue ACE activity was also studied, and from the result obtained (Figure 1), induction of diabetes significantly increased ($P < 0.05$) lung ACE activity (1.37 ± 0.03 $\mu\text{mol/min ml}$) compared to rats in control group (1.09 ± 0.10 $\mu\text{mol/min ml}$). This decreased significantly ($P < 0.05$) when rats were given insulin (0.59 ± 0.04 $\mu\text{mol/min ml}$), 250 mg/kg (0.95 ± 0.03 $\mu\text{mol/min ml}$) and 100 mg/kg (1.02 ± 0.01 $\mu\text{mol/min ml}$) of the extract.

The ACE activity in the kidneys (1.36 ± 0.03 $\mu\text{mol/min ml}$) and heart (1.38 ± 0.01 $\mu\text{mol/min ml}$) of untreated diabetic rats increased significantly ($P < 0.05$) when compared to the kidneys (0.68 ± 0.04 $\mu\text{mol/min ml}$) and heart (0.81 ± 0.04 $\mu\text{mol/min ml}$) of control rats. Treatment of diabetic rats with insulin significantly ($P < 0.05$) reduced kidney (1.05 ± 0.02 $\mu\text{mol/min ml}$) and heart (0.65 ± 0.02 $\mu\text{mol/min ml}$) ACE activity than rats treated with the extract at both doses. The decrease in ACE activity in heart of rats given the extract at 100 (0.99 ± 0.01 $\mu\text{mol/min ml}$) and 250 mg/kg (0.85 ± 0.02 $\mu\text{mol/min ml}$) was dose-dependent.

DISCUSSION

The measurement of blood glucose is the most important biological marker used in the diagnosis and monitoring of diabetes mellitus both in clinical and experimental settings (Mayfield, 1998). The significant increase in blood glucose level upon treatment with streptozotocin is evident that diabetes was induced in the rats. The diabetic effect of streptozotocin has been attributed to a specific cyto-toxic action mediated by hydroxyl radical generation in pancreatic β -cell, which damages a large number of β -cells resulting in a decrease in endogenous insulin release (Szkudelski, 2001). These result in elevated blood glucose within a short period of time after streptozotocin

administration (Milagro and Martinez, 2000; Haidari et al., 2012).

In the present study, it was observed that the combined extract of *V. amygdalina* and *O. gratissimum* reversed this (Table 1). Our result is in consonance with reports by Asuquo et al. (2010) and Okokon et al. (2013) who demonstrated the hypoglycemic effect of the combined extract. Although the mechanism of action of the extract was not explored, studies have shown that the causes and sites of intervention in the biochemical process of diabetes is diverse (Larner, 1985). One of such is that blood glucose lowering drugs may act through stimulation of synthesis and/or release of insulin from the beta-cells of the pancreatic islets. They may also increase sensitivity of receptors to insulin, insulinase inhibiting effect, and stimulation of peripheral tissues uptake of glucose cannot be ruled out (Szkudelski, 2001). Also, it has been reported that some herbs or plants reduce absorption of carbohydrates in the digestive system causing progressive entry of glucose into the blood and prevent sudden increase in blood glucose after food intake (Jenkins et al., 1980).

In other studies, the hypoglycaemic and hypolipidemic activities of some plants have been attributed to their fiber and antioxidant contents (Lemhadri et al., 2007). Recently, Ong et al. (2011) demonstrated that *V. amygdalina* decreased blood glucose by increasing GLUT 4 translocation and inhibiting hepatic glucose-6-phosphatase. Thus, it is possible that the two extracts may have exerted their hypoglycemic effects by utilizing one or more of the above mechanisms. A combination of these mechanisms could have resulted in the significant hypoglycemic activity observed in this study, which is likely to be sustained and better than that of a single extract.

The high concentration of TAG, TC and LDL-C observed in diabetic rats compared to control rats in this study is consistent with reports of several studies (Pari and Latha, 2002; Akah et al., 2004; Nwanjo and Oze, 2007; Ayinla et al., 2011) demonstrating that a rise in glucose level on induction of diabetes, results in a corresponding increase in plasma lipids. Hyperlipidemia is a recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, triglycerides, phospholipids and other lipoproteins (Segal et al., 1984; Ayinla et al., 2011). It has been reported that elevated serum lipids in diabetes is due to the increased mobilization of free fatty acids from peripheral fat depots as a result of inhibition of the hormone sensitive lipase (Sharma et al., 2010). The excess fatty acids produced are converted into phospholipids and cholesterol, which together with excess triacylglycerols formed at the same time in the liver are discharged into the blood in form of lipoproteins. Thus, the marked hyperlipidemia observed in diabetic rats may be regarded as a consequence of uninhibited actions of lipolytic hormones in fat depots (Goodman and Gilman, 1985). Treatment of diabetic rats

with the combined extract of *V. amygdalina* and *O. gratissimum* caused a significant ($P < 0.05$) decrease in serum and liver lipids, showing its hypolipidemic effect.

The results of this study support earlier reports (Akah et al., 2004; Nwanjo, 2005; Nwanjo and Oze, 2007; Akah et al., 2009; Ekeocha et al., 2012; Ayinla et al., 2011) and could be related to the presence of alkaloids, saponins, flavonoids and polyphenols (Ayoola et al., 2008; Akinmoladun et al., 2007) known to reduce serum lipid level in animals (Ezekwe and Obioha, 2001).

Lipid peroxidation is a characteristic of diabetes. The increase of free radicals in diabetic condition is suggested to be due to increased lipid peroxidation (Kakkar et al., 1995; Baynes and Thorpe, 1999) and damage of antioxidant defence system as a result of increased production and/or decreased destruction by non-enzymic and enzymic catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) (Moussa, 2008). Elevated lipid peroxide commonly based on concentration of TBARS and decreased activity of antioxidant molecules in diabetic rats could probably be associated with oxidative stress and decreased antioxidant defence potential (Koshio et al., 1994; Rosa et al., 2003). Results of the present study is consistent with previous findings (Chis et al., 2009; Bhaskar and Kumar, 2012) demonstrating a significant ($P < 0.05$) increase in TBARS level and concomitant decrease in SOD and catalase in diabetic rats compared to control rats. SOD protects tissues against oxygen free radicals by converting the superoxide radical into hydrogen peroxide and molecular oxygen, while catalase catalyses the detoxification of hydrogen peroxide, preventing damage to cell membranes and other biological structures (Chis et al., 2009). Administration of combined extract of *V. amygdalina* and *O. gratissimum* reduced TBARS to normal levels, while SOD and catalase increased in a dose-dependent manner. Our result supports several studies demonstrating the antioxidant properties of the extracts (Nwanjo, 2006; Akinmoladun et al., 2007; Ayoola et al., 2008; Owolabi et al., 2008; Ong et al., 2011), which has been attributed to the presence of flavonoid and polyphenols in both plants known to possess antioxidant activities (Akah and Okafor, 1992; Ong et al., 2011).

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of a known disease and determine the effects of potentially hepatotoxic drugs (Harris, 2005). The significant ($P < 0.05$) increase in the serum AST observed in diabetic rats (Table 5) is consistent with studies by Nwanjo (2007), indicating liver damage as a result of an increase in hepatic glucose output and a decline in hepatic insulin sensitivity (Harris, 2005). The decrease in AST in rats given the combined extract indicates the possible hepatoprotective effect of the plant extracts (Iwalokun et al., 2006).

With respect to kidney function, the high serum urea

levels in diabetic rats compared to control is consistent with studies by Atangwho et al. (2007) and proves that diabetes affects kidney function. This has been shown to be due to the stimulation of gluconeogenesis as alternative glucose supply route as a result of insulin deficiency. Gluconeogenesis is sustained by increased proteolysis which releases glucogenic amino acids that are deaminated in the liver resulting in high urea levels (Robinson and Johnston, 1997). The significant reduction in urea after treatment can be attributed to the ability of the extract to reduce glucose concentration and thus increase insulin effect causing a decline in proteolysis (Robinson and Johnston, 1997).

Results of the present study, which clearly demonstrates that lung, heart and kidney ACE activity is elevated in STZ-induced diabetic rats, supports earlier reports (Erman et al., 1998; Hosseini et al., 2007) that ACE levels increase in serum of diabetic rats. It is well established that the renin-angiotensin aldosterone system (RAAS) is involved in diabetic nephropathy, due to high ACE levels, abnormal aldosterone to renin ratio and altered angiotensin II sensitivity (Lely et al., 2007).

Although it has been speculated that diffuse vascular damage (Lieberman, 1975), and increased shedding from the endothelium in diabetes mellitus may release ACE in the blood stream (Van dyk et al., 1994), the precise underlying mechanism for the increased ACE activity in diabetes mellitus remains to be clarified. The significant ($P < 0.05$) decrease in lung, heart and kidney ACE activity in rats given 250 mg/kg aqueous extracts compared to diabetic rats, may be attributed to its ability to reduce blood sugar levels.

Also, the extract may be a good ACE inhibitor, as it has been shown that blocking the renin-angiotensin system with an ACE inhibitor slows the progression of diabetic nephropathy by a mechanism independent of their anti-hypertensive property (Lewis et al., 1993; Nielsen et al., 2009).

In conclusion, this study has demonstrated that a combination of the aqueous leaf extract of *V. amygdalina* and *O. gratissimum* induces significant reductions in the blood glucose, lipoprotein and ACE levels of streptozotocin-induced diabetic rats. It also exhibits antioxidant properties and could prevent impairment of the kidneys and liver in the diabetic state.

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