Biochemical and antioxidants activity of crude, methanol and n-hexane fractions of *Vernonia calvoana* on streptozotocin induced diabetic rats

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This study is aimed at evaluating the biochemical effects and antioxidants activity of extracts of *Vernonia calvoana* Hook. f (V.C) on STZ induced diabetic rats. Thirty-six rats weighing (100 to 150 g), were divided into 6 groups of 6 animals each. Groups 1 and 2 representing normal and diabetic controls (NC and DC), respectively, receiving placebo, while groups 3 to 6 represented diabetic treated, receiving 500 mg/kg body weight (b.w) metformin, 400 mg/kg b.w. crude, n-hexane and methanol fractions of V.C, respectively. Treatment with drug and extracts of V.C showed a decrease in fasting blood glucose (FBG) in all experimental groups and was significant (p<0.05) on the 7th day of the experimental period, compared to diabetic control. Progressive increase in body weight was observed in all experimental groups compared to DC group. A significant (p<0.05) increase in glutathione peroxidase (GPX) and catalase (CAT) activities were recorded in all experimental treated animal compared to DC and NC. Malondialdehyde (MDA) concentration was observed to decrease significant (p<0.05) in all experimental groups compared to DC. Histopathologically, the changes in pancreatic integrity were consistent with that of biochemical findings. It may be concluded that, extracts of V.C possess potent ameliorative activity against STZ-induced diabetes, via a potential free radical mopping activity.

**Key words:** *Vernonia calvoana*, extracts, diabetes mellitus, antioxidants.

**INTRODUCTION**

Deep rooted in the cultures of rural dwellers is the use of plants and plant-parts as first point of call for both their daily primary health care and nutritional needs. According to World Health Organization (WHO), about 65 to 80% of the world’s population in developing countries depends essentially on plants and plant derived compounds for their primary healthcare and as source of nutrition (WHO, 2014).

Report by WHO globally, estimated that approximately 5 to 8% of the global population is affected by diabetes mellitus (Chakraborty and Rajagopalan, 2002). Diabetes now is becoming the third “killer” of mankind along with cancer, cardiovascular and cerebrovascular disease (Donga et al., 2011). It has also been predicted that by
the year 2025, more than 75% of people with diabetes will reside in developing countries (King, 2012). Significant amount of synthetic antidiabetic drugs like glybenclamide, metformin, and thiazolidiones are well known today not only to be expensive but also produces serious side effects (Venkatesh et al., 2003). Therefore, there has been a growing interest in the ethnobotanical approach to screen the anti-diabetic properties of plants traditionally used in our locality and other parts of the world.

Vernonia (Asteraceae) plant species is the largest genus in the tribe Vernoniae, with close to 1000 species (Keely and Jones, 1979). The genus Vernonia has several species, some which are useful as food, medicinal agents and industrial raw materials. Vernonia calvoana and Vernonia colorata species are both eaten as leafy vegetables (Burkill, 1985; Iwu, 1993).

V. calvoana (Hook f) belongs to the family of Asteraceae. It is found locally in mountainous and high plateau regions of West, Central, East and Southern Africa. The plant is also known as Vernonia hymenolepis A. Rich, Vernonia leucocalyx or Baccharoides calvoana. In English, it is called sweet bitter leaf or bitter leaf. In France, it is called Vernonia douce or Vernonia. The Cameroonian call it Bayangi or Ndole. It is also known as “Ekeke leaf” among the indigenous people of the central senatorial district of Cross River State of Nigeria (Igile et al., 2013). It serves as a green leafy vegetable and is also used for ethno-medical purposes in Nigeria and Cameroun (Focho et al., 2009). It is popularly eaten raw and fresh as a local delicacy with or without palm oil in pepper sauce because the vegetable imparts a sweet taste like sugar in the tongue after its consumption. It serves as a component of traditional salad among the indigenous consumers. It may also be cooked in native soups. Its consumption is based on the belief that the plant is use in the management and cure of heart diseases, blindness, diabetes, malaria, stomach ache, as an anti-helminthic agent, and to prevent constipation. The vegetable is less bitter than the sister plant (Vernonia amygdalina), and yet both plants are used for the same ethno-medical purposes both as food and for traditional treatment of diseases in some part of south-south of Nigeria (Igile et al., 2013). Preliminary pharmacological studies carried out in experimental models have validated the plant to have hypoglycemic and hypolipidemic activity (Iwara et al., 2015). Chemical evaluations of these plants by Igile et al. (2013) have revealed high levels of antioxidant vitamins (A, C, E and B-complex), mineral elements (Fe, Se, Zn, Cu, Cr and Mn) and phytochemical compounds (polyphenols, flavonoids and tannins) in the leaves of V. calvoana. These antioxidant and phytochemicals compounds have been reported widely to ameliorate free radical mediated disease like cancer, mutagenesis, cardiovascular disease, and diabetes by neutralizing the ROS generated (Atangwho et al., 2013).

The observed pharmacological activities of this plant extract may be due to the presences of bioactive phytochemicals. Therefore, this study was designed to investigate the biochemical effect and anti-oxidant activity of crude extract and fractions of V. calvoana in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

Sample collection and preparation

Fresh leaves of V. calvoana were harvested from a farm in Ugep, in Yakurr L.G.A of Cross River State, Nigeria. The leaves were collected in the early hours of the day, cleaned and air dried for 7 days after which they were ground into powder form. A measured quantity of 5 kg of powder leaves were extracted via cool maceration in 8 L of 80% ethanol for 48 h. The extract was further double filtered with chess cloth, then with filtered paper (Whatman 4 filtered paper) and the residue obtained was further extracted with 4 L of 80% ethanol. The filtrate was then concentrated at 45°C in rotary evaporator to 10% volume and then to complete dryness using water bath yielding 310.3 g (6.2%) of crude extract. The crude extract obtained was subjected to fractionation.

Fractionations of plant extract using column chromatography

The crude extract (251.8 g) was chromatographically eluted with two different solvents: n-hexane and methanol in a column packed with silica gel of mesh 60 to 120. The fractions were collected and evaporated in rotary evaporator at 50°C to 10% of its original volume and further evaporated to paste form in a water bath at 50°C. The percentage yield for the fractions were 12 g (4.8%) methanol fraction and 20 g (8.10%) n-hexane fraction. The fractions and the remaining crude extract were stored in a freezer at -4°C for further experiments.

Acute toxicity testing

Acute toxicity level of the fractionated extracts was carried out in mice (Mus Musculus) to determine the dose levels to be administered to the experimental animal using Lorke’s method (Lorke, 1983).

Animal handling/design

Thirty-six Wistar rats of both sexes weighing 100 to 150 g were obtained from the animal house of the Department of Zoology and Environmental Biology, University of Calabar, Calabar. The animals were allowed to acclimatize for three weeks in the animal house of the Department of Biochemistry and were housed in well ventilated cages (wooden bottom and wire mesh top), and kept under normal environmental conditions of room temperature and relative humidity. Administration of extract was twice daily in 6 h cycle. The animals were divided into six groups of six animals each (Table 1). The protocol was in accordance with the guidelines of the National Institute of Health (NIH) publication (1985) for laboratory Animal Care and Use and approved by the College of Medical Sciences Animal Ethics Committee, University of Calabar, Nigeria (Atangwho et al., 2013).

Induction of diabetes

Diabetes was induced with 45 mg/kg body weight of STZ in 0.1 M
Table 1. Experimental design.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animal</th>
<th>Treatment</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6</td>
<td>Placebo</td>
<td>0.2 ml 10% DMSO</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6</td>
<td>Placebo</td>
<td>0.2 ml 10% DMSO</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>6</td>
<td>Metformin</td>
<td>500 mg/kg</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>6</td>
<td>Methanol fraction</td>
<td>400 mg/kg</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>6</td>
<td>n-Hexane fraction</td>
<td>400 mg/kg</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>6</td>
<td>Crude fraction</td>
<td>400 mg/kg</td>
</tr>
</tbody>
</table>

sodium citrate buffer (pH 4.4). Animals with fasting blood glucose >7.8 mmol/l or > 180 mg/dl were enrolled for the study (Ebong et al., 2008).

Experimental protocol

Animals were grouped as shown in the aforementioned scheme and also accordingly treated with extracts of V.C and metformin. The dosages of the plant extracts were as determined from preliminary work in our laboratory (Iwara et al., 2015). Metformin (Glucophage) (500 mg/kg b.w. S.C.) was as previously used by Atangwho et al. (2013). The plant extracts and glucophage was administered via oral gastric intubation, twice per day (10.00 am: 4.00 pm). Treatment lasted for 21 days and throughout this period animals were maintained on pallets prepared with Growers feed from Vital Feeds, Jos, Plateau state, Nigeria, and tap water. Both feed and water were provided ad libitum.

Measurement of fasting blood glucose and body weight

Fasting blood glucose was determined at interval of 7 days during the 3 week experimental period using glucometer (one touch). Body weight of animals was determined also at interval of 7 days using weighing balance.

Collection of samples for analysis

After 21 days experimental period, food was withdrawn from the rats and fasted overnight with access to water. The rats were then anaesthetized over chloroform vapor and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles, and emptied into EDTA bottle, allowed for 2 h stored in a refrigerator at 4°C. The refrigerated blood sample was then centrifuged at 3000 rpm for 10 min to recover the plasma from cells. Plasma was separated with sterile syringes and needle and stored frozen until used for biochemical analysis.

Pancreas was removed and blotted with Whatman No. 1 filter paper to clean the excess blood on the organs, and then weighed using weighing balance. Thereafter, a portion of the tissue was sliced and suspended in 10% fixative (formal saline) for histological analysis.

Biochemical analyses

Analytical kits for glucose analysis were purchased from Agappe diagnostics. GPX, MDA and CAT kit where purchase from Biovision incorporated.

Histopathology

The histological examination of the pancreas of the induced models and the control was carried out using differential staining procedure described by Drury and Wallington (1967).

Statistical analysis

The results were analyzed for statistical significance by one way analysis of variance (ANOVA) with a post hoc Dunnet at (p < 0.05 t) using SPSS software and Microsoft Excel. All data were expressed as mean ± standard error of mean (SEM; n = 6 replications).

RESULTS

Effect of crude extract, methanol and n-hexane fractions of V.C on blood glucose

Hourly changes in fasting blood glucose for 4 h, changes in plasma blood glucose (PBG) and weekly fasting blood glucose (FBG) were determined in this study during the 21 days experimental period. The results obtained are presented in Figures 2 and 3 and Tables 2 and 3. From the result, the PBG (Figure 1) of diabetic control (DC) groups was observed to be significantly (p<0.05) increased, compared to the normal control (NC) group. Upon treatment with test drug/sample (Metformin, crude extract, methanol and n-hexane fractions of V.C), significant (p<0.05) decrease in PBG was observed in all treated groups, compared to both DC and NC groups; with crude extract, methanol and n-hexane fractions treated groups showing close activity to that of metformin treated groups. Also from Table 2, the weekly FBG levels of diabetic control groups were observed to be significantly (p<0.05) increased, compared to NC groups at the beginning of the experiment with constant level till the end of the experimental period. However, on treatment with test drug and the fractions, decrease in FBG was observed in all experimental groups. The decrease was more significant on the 7th day of the experimental period, compared to the diabetic control, and thereafter increased on day 14, and then reduced on day 21. Moreso, the hourly changes in FBG (Table 3) within 4 h of the first day of experiment showed increase in the glucose levels within 1 h in the DC and methanol
Table 2. Effect of crude extract, methanol, n-hexane fractions of V.C leaves and metformin on weekly Fasting blood glucose of STZ-induced Diabetic rats.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Basal (mg/dl)</th>
<th>Day 7 (mg/dl)</th>
<th>Day 14 (mg/dl)</th>
<th>Day 21 (mg/dl)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>81±7.31</td>
<td>112±5.2</td>
<td>100±4.4</td>
<td>72±2.7</td>
<td>-12.5</td>
</tr>
<tr>
<td>DC</td>
<td>284±2.1*</td>
<td>393±8.5</td>
<td>395±10.7*</td>
<td>387±7.4*</td>
<td>26.6</td>
</tr>
<tr>
<td>Met</td>
<td>278±4.6*</td>
<td>226±8.2*</td>
<td>195±4.4*</td>
<td>106±3.1*</td>
<td>-162</td>
</tr>
<tr>
<td>V.C Crude</td>
<td>254±4.4*</td>
<td>192±9.6*</td>
<td>259±8.8*</td>
<td>267±1.3*</td>
<td>4.7</td>
</tr>
<tr>
<td>V.C Met</td>
<td>252±5.6*</td>
<td>67±10.8*</td>
<td>190±6.4*</td>
<td>155±1.8*</td>
<td>-62.6</td>
</tr>
<tr>
<td>V.C-Hex</td>
<td>285±9.1*</td>
<td>183±7.3*</td>
<td>352±1.2*</td>
<td>230±1.2*</td>
<td>-23.9</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ±SEM (n= 6). *Significantly different from NC at p<0.05. a=p<0.05 vs. DC.

Table 3. Effect of crude extract, methanol, n-hexane fractions of V.C leaves and metformin on hourly Fasting blood glucose of STZ-induced Diabetic rats.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Basal (mg/dl)</th>
<th>1 h (mg/dl)</th>
<th>2 h (mg/dl)</th>
<th>4 h (mg/dl)</th>
<th>% Change in FBG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>81±7.31</td>
<td>102±1.7</td>
<td>95±2.3</td>
<td>72±2.7</td>
<td>-12.5</td>
</tr>
<tr>
<td>DC</td>
<td>284±2.1*</td>
<td>550±2.6*</td>
<td>450±4.3*</td>
<td>430±3.2*</td>
<td>33.9</td>
</tr>
<tr>
<td>Met</td>
<td>278±4.6*</td>
<td>184±4.0*</td>
<td>160±1.7*</td>
<td>159±2.1*</td>
<td>74.8</td>
</tr>
<tr>
<td>V.C Crude</td>
<td>254±4.4*</td>
<td>284±3.8*</td>
<td>250±3.2*</td>
<td>253±1.8*</td>
<td>3.9</td>
</tr>
<tr>
<td>V.C Met</td>
<td>252±5.6*</td>
<td>483±4.6*</td>
<td>485±2.1*</td>
<td>281±2.4*</td>
<td>10.3</td>
</tr>
<tr>
<td>V.C-Hex</td>
<td>285±9.1*</td>
<td>258±7.2*</td>
<td>258±2.5*</td>
<td>272±3.1*</td>
<td>-4.7</td>
</tr>
</tbody>
</table>

NC: Normal control, DC: diabetic control, MET: metformin, V.C CRUDE: Vernonia calvoana crude, V.C MET: Vernonia calvoana metformin, V.C_nHEX: Vernonia calvoana hexane. Values are expressed as Mean ± SEM (n= 6). *Significantly different from NC at p<0.05. a=p<0.05 vs. DC.

Figure 1. Plasma glucose concentrations of the different experimental groups. Values are expressed as Mean±SEM (n= 6). *Significantly different from NC at p<0.05. a=p<0.05 vs. DC, b=p<0.05 vs. VC_MET.
fraction treated experimental groups. The NC, metformin and n-hexane fractions of V.C showed marked reduction in FBG 1 h after first administration.

**Effect of crude extract, methanol and n-hexane fractions of V.C leaves on body weight changes**

The results of changes in the body weight of experimental animal after 21 days period are presented in Table 4. Observed from these result was a significant (p<0.05) reduction in body weight of DC, compared to NC groups. On treatment with test drugs/fractions after 7th day period, progressive increase in body weight was observed in all experimental groups compared to the DC group, and the steady body weight increase was maintained till the 21st day. However, at 14th day period, the DC groups showed an increased body weight that was also sustain till the last day.

**Effect of crude extract, methanol, n-hexane fractions of V.C leaves and metformin on oxidative oxidative stress marker and antioxidant enzyme**

The levels of antioxidant enzymes and oxidative stress maker are shown in Figures 2, 3 and 4. Results obtained showed a significant (p<0.05) decrease in the activities of glutathione peroxidase (GPX) and catalase (CAT) (Figures 2 and 3) in DC, compared to NC. A significant (p<0.05) increase in GPX and CAT activities were recorded on administration of the diabetic rats with n-hexane and methanol fractions of V.C, compared to DC and NC. Also, an insignificant (p>0.05) increase in the concentration of MDA was observed in DC, compared to the NC (Figure 4). On administration of V.C crude extract, methanol, n-hexane fractions and metformin, the MDA concentrations was observed to reduce with the n-hexane fraction showing the most significant (p<0.05) reduction.

**Effect of treatment on the histology of pancreatic tissues**

Presented in Figures 3a, b, c, d, e and f are the cellular architecture of pancreas. Photomicrograph of a section of the pancreas of NC group (Figure 3a) showed compact islets consist of round to oval, well circumscribed collections of endocrine cells. The cells have uniform round nuclei with coarsely clumped chromatin and inconspicuous nucleoli. The cytoplasm is pale. The cells are separate by small capillaries into lobules. The insulin producing cells are located centrally and the glucagon cells peripherally located. The surrounding pancreatic acinar have abundant cytoplasm and basally located nuclei. Induction for diabetes (DC group, Figure 3b), showed a compact islet surrounded by pancreatic acinar cells. The islet cells are oval to round separated by blood capillaries. The cells are sparsely populated with scanty cytoplasm and clumped chromatin pattern. The acinar are unremarkable. On treatment with metformin (Figure 3c) showed a hyperplasia of islet of Langerhans surrounded by pancreatic acinar cells. The islets are compact consisting of uniform round to oval cells with regular outline, scanty cytoplasm and clumped chromatin pattern. In the methanol treated group (Figure 3d), the islets consisted of a densely packed round to oval, collections of deeply stained endocrine cells; having a round nuclei with coarsely clumped chromatin and inconspicuous nucleoli. The cells are separate by small capillaries. The surrounding pancreatic acinar and lined by tall cuboidal cells with basally located. Also, the n-hexane treated group (Figure 3e) showed islets consisting of round to oval, collections of sparsely populated endocrine cells. These cells are loosely packed with round nuclei having coarsely clumped chromatin and inconspicuous nucleoli. The cells are separate by small capillaries into lobules. There cytoplasm are pale while that of the crude extract (Figure 3f) was observed to consist of an islet having a sparsely populated endocrine cells. The cells have oval to round nuclei with coarse chromatin patterns and are separated by thin walled capillaries. These cells are more at the

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Basal (g)</th>
<th>Day 7 (g)</th>
<th>Day 14 (g)</th>
<th>Day 21 (g)</th>
<th>% Change in BW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>88±1.7</td>
<td>112±8.5</td>
<td>131±10.2</td>
<td>119±6.6</td>
<td>26</td>
</tr>
<tr>
<td>DC</td>
<td>145±7.4</td>
<td>138±6.9</td>
<td>124±3.7</td>
<td>138±2.8</td>
<td>-5.1</td>
</tr>
<tr>
<td>Met</td>
<td>101±3.8</td>
<td>106±3.8</td>
<td>124±4.6</td>
<td>148±9.7</td>
<td>31.8</td>
</tr>
<tr>
<td>V.C Crude</td>
<td>108±5.2</td>
<td>108±9.0</td>
<td>123±8.4</td>
<td>128±11.7</td>
<td>15.6</td>
</tr>
<tr>
<td>V.C Met</td>
<td>109±2.8</td>
<td>107±0.8</td>
<td>117±0.9</td>
<td>125±2.7</td>
<td>12.8</td>
</tr>
<tr>
<td>V.C Hex</td>
<td>122±5.5</td>
<td>112±3.8</td>
<td>131±6.7</td>
<td>128±2.6</td>
<td>4.7</td>
</tr>
</tbody>
</table>

NC: Normal control; DC: diabetic control; MET: metformin; V.C CRUDE: Vernonia calvoana crude; V.C MET: Vernonia calvoana metformin, V.C_nHEX: Vernonia calvoana hexane. Values are expressed as Mean ± SEM (n= 6). *Significantly different from NC at p<0.05. **p<0.05 vs. DC.
central portion and the surrounding acinar are closely packed and lined cuboidal epithelium.

DISCUSSION

From time immemorial, nature has been a source of medicinal agents. Significant number of modern drugs have been isolated and characterized from natural sources. Medicinal plants have been used for centuries as remedies for human and animal diseases as they contain phyto-chemicals of therapeutic value. Green plants are known to represent a reservoir of effective chemotherapeutic agents with more systemic and easily biodegradable potentials (Atangwho et al., 2013).

The administration of streptozotocin at recommended therapeutic dose to animals induces a response in the concentration of glucose in the blood with an accompanying change in the insulin concentration as well as sequential change in the architecture of the beta cells (Mythili et al., 2004). Streptozotocin, being a diabetogenic agent, inhibits insulin secretion and causes a state of insulin-dependent diabetes mellitus by selectively causing necrosis of the pancreatic beta cell, and this can be related to alkylating potential of STZ. Consequently, in the present study, it was observed that injection of 45 mg/kg b.w of STZ caused an increase in fasting and plasma blood glucose of diabetic control (DC) compared to that of normal; with result of the test extracts comparing favorable with metformin (a known standard diabetic drug). These results were in agreement with the earlier report by Iwara et al. (2015) on the hypoglycemic and hypolipidemic potentials of V. calvoana in alloxan-induced diabetic rats. However, from the observed results, it may be assumed that the n-hexane extract of V. calvoana is less likely to have hypoglycemic activity;
thus giving it more desirable antidiabetic features of medicinal plants. It can also be further deduced from the present study that both the methanol and n-hexane fractions of *V. calvoana* contain long-term glycemic constituents which will be of great use for future studies.

Tissue wasting is one of the observable features of untreated diabetic condition in experimental rat models (Ahmed, 2005). This occurs as a result of lack of uptake of glucose by the tissues which serve as the primary source of energy for the body due to insufficient insulin that signals glucose uptake in the body. As a result, the body starts burning fat and muscles for energy production thus leading to lost in weight. In the present study, a significant decrease in body weight of diabetic control animals, compared to normal control, was observed; showing a clear indication of the deterioration of the glucose control mechanism, which progresses in stages and would probably climax in the death of the animal if left untreated (Atangwho et al., 2012). Upon treatment of the diabetic rats with extracts of *V. calvoana* and metformin, a significant increase in body weight was observed. This implies that treatment with experimental drugs helps to enhance availability of glucose to the tissues, both for supply energy and to build tissue materials needed for growth.

Increased oxidative stress has been postulated to play a key role in the pathogenesis of diabetes mellitus associated complications like neuropathy, nephropathy,
Figure 2c. Malondialdehyde concentrations of the different experimental groups. Values are expressed as mean ± SEM (n=5). *Significantly different from NC at p<0.05. a p<0.05 vs. DC, b p<0.05 vs. MET, c p<0.05 vs. crude VC. NC: Normal control, DC: diabetic control, MET: metformin, V.C CRUDE: Vernonia calvoana crude, V.C MET: Vernonia calvoana metformin, V.C nHEX: Vernonia calvoana hexane.

cardiomyopathy, and retinopathy (Williams et al., 2013). Basically, hyperglycemic conditions are associated with elevated reactive oxygen species (ROS) production, predominantly through mitochondrial electron transport chain and nicotinamide adenine dinucleotide phosphate oxidase (Ray and Shah, 2005). The possible sources of ROS include autoxidation of glucose, shifts in redox balance, decreased tissue concentration of glutathione and vitamin E, as well as impaired activity of superoxide dismutase (SOD) and catalase (CAT). Oxidative stress has been widely established as a major contributory factor in the development and progression of diabetes and its complications. It has been suggested that insulin resistance may be accompanied by intracellular production of free radicals. Thus, a vicious cycle between hyperinsulinemia and free radicals could be operating in the early stages of diabetes pathogenesis. Insulin resistance induced elevated plasma free radicals, in turn,
Figure 3. (a, b, c, d, e, f) Photomicrographs of pancreas (normal and diabetic control) and (metformin and methanol group) (X 400). H&E = Haematoxilin and Eosin. IL=islet of Langerhans, IC=islet cell, PC=pancreatic cell, A=acinar, N=nucleus. Photomicrographs of pancreas (normal and diabetic control) and (n-hexane and crude extract group) (X 400). H&E = Haematoxilin and Eosin, G=Gomeri stains. IL=islet of Langerhans, IC=islet cell, PC=pancreatic cell, A=acinar, N=nucleus.
may cause a deterioration of insulin action, with hyperglycemia being a contributing factor (Ceriello, 2000). Other mechanisms by which increased oxidative stress is involved in the diabetic complications include activation of several transcription factors, protein kinase C, and advanced glycosylated end products (AGEs). In the present study, decrease in antioxidant enzymes, including glutathione peroxidase (GPx) and CAT activities, and increase in MDA concentration recorded for DC were reversed upon treatment with the leaves’ extracts and standard drug. This effect may be attributed to the presence of bioactive components reported in this study for this plant such as 2-furamethanol, 2-tridece-1-ol, phytol, n-hexadecanoic acid, 9, 12, 15-octadecatrien-1-ol for the n-hexane extract and 2-butanol, 1-hydroxy-3-methyl-2-butanoic acid, hexadecanoic acid, oleic acid, e-11-teradecenoic acid and cyclohexane propanol for the methanol fraction. Probably, these compounds may by reacting with a free radical, donates hydrogen atoms with an unpaired electron (H∙), converting free radicals into less reactive species (Iwara et al., 2016). This may also contributed to the observed antioxidant activities reported in this study for VC leaves’ extracts. The observation agrees with the earlier reports by Igile et al. (2013) and Iwara et al. (2015).

Streptozotocin is known to induce chemical diabetes (type 1) by selective destruction of pancreatic beta cells through three processes, including deoxyribonucleic acid alklylation, nitric oxide production and free radical generation (Szkudelski, 2001). The selective pancreatic beta cell toxicity of s STZ and the resulting diabetic metabolic state are clearly related to the glucose moiety in its chemical structure, which enables streptozotocin to enter the beta cell via the low affinity Glut 2 glucose transporter in the plasma membrane (Elsner et al., 2000). This condition was observed in this study, as diabetic control rat pancreas showed a compact islet surrounded by pancreatic acinar cells. The observation is consistent with the literature reports on STZ-induced pancreas damage (Noor et al., 2010). However, these lesions were slightly reversed or ameliorated after treatment with met and extracts of V. calvoana indicating a recovery effect. The recovery may be attributed to the phytochemicals that has been reported by Igile et al. (2013) and in this research. Moreover, it was observed in this study that components in the extract of methanol fractions appears to mimic the standard drugs in the following parameters plasma blood glucose, fasting blood glucose, body weight changes, GPX and MDA and may follow the same mechanism of action.

Conclusion

Conclusively, findings in this study suggests potent ameliorative activity against STZ-induced diabetes, potentially due to free radical mopping activity of the extract and thus ameliorate metabolic complications of diabetic state.

Conflict of Interests

The authors have not declared any conflict of interests.

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