European Journal of Medicinal Plants
6(3): 143-153, 2015, Article no.EJMP.2015.050
ISSN: 2231-0894

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Immunological and Haematological Disturbances in Diabetes Mellitus: Modulatory Role of Diets Containing *Vernonia amygdalina* Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author HDA was involved in conception, design, acquisition of data and drafting the manuscript. Author IFU was involved in analysis and interpretation of data, revising the draft copy and approving the final copy to be published taking into consideration the accuracy and integrity of all parts of the work. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/14313
Editor(s):
(1) Thomas Efferth, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry Johannes Gutenberg University, Germany.
(2) Marcello Iriti, Faculty of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Yakubu Abdulrahaman, Department of Haematology, Faculty of Medical Laboratory Science, Usmanu Danfodiyo Univesity, Sokoto. North West Nigeria.
(2) Anonymous, USA.
(3) Anonymous, Cameroon.
(4) Anonymous, Iran.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=913&id=13&aid=7660

Original Research Article

Received 26th September 2014
Accepted 20th December 2014
Published 7th January 2015

ABSTRACT

Aim: The aim of this study was to determine some makers of immunology and haematology in Streptozotocin -induced diabetic wistar rats consuming *Vernonia amygdalina* leaf diets in order to evaluate the involvement of the diets in the management of immunological and haematological complications among diabetics.
Design and Methodology: Fifty albino wistar rats were divided into five groups with 10 rats in each group. Group 1 (normal control) was fed with control diet; Group 2 (diabetic control) was fed with control diet, Group 3 and 4 (diabetic treated with diets) were fed with *Vernonia amygdalina* leaf

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diet at 5% and 7.5%, respectively. Group 5 (diabetic treated with insulin) was fed with control diet and administered insulin. Feed and water were given *ad- libitum* for 28 days. Full blood count (FBC) was determined using automated haematology analyzer, KX2IN (non-cyanide hemoglobin analysis method. The CD_4^+ lymphocyte was estimated by flow cytometry using the cyflow automated cell counter (Parlec, Germany).

Results: Results *showed* that diabetic rats consuming *Vernonia amygdalina* had significant ($P < 0.5$) increase in the RBC, haemoglobin, and lymphocyte counts relative to the diabetic control. Diabetic rats consuming *Vernonia amygdalina* had significant ($P < 0.5$) reduction in the level of WBC, platelets, neutrophil, and CD_4^+ cell count relative to the diabetic control. The results for *Vernonia amygdalina* diets were similar to insulin on the measured parameters and their levels were not significantly different ($P > 0.5$) when compared to the normal control.

Conclusion: We concluded that consumption of diets containing *Vernonia amygdalina* leaves might have positive effect on the immunological and haematological abnormalities associated with diabetes mellitus.

Keywords: Diabetes; diet; Vernonia amygdalina; immunological; hematological.

1. INTRODUCTION

Diabetes mellitus (DM) is a major global health problem [1,2]. According to estimates of the World Health Organisation (WHO), the number of people suffering from DM worldwide is increasing at an alarming rate. There were 346 million people suffering from diabetes worldwide in 2011 [3]. It is predicted that about 366 million people are likely to be diabetic by the year 2030 [4]. Reports show that low and middle-income countries will bear the brunt of the increase and that Africa will contribute significantly to the rise [4]. In Africa the prevalence of DM is comparatively high in young to middle-aged people unlike the West where the older are most affected. This may have long-lasting adverse effects on the nation's health and economy. Cure eludes physicians and many sufferers cannot meet the cost of conventional drugs. Therefore, it has become imperative to investigate alternative sources of medicament, especially those that are cheap and easily sourced. In most developing countries attention has been focused on medicinal plants. Studies on medicinal plants however have concentrated on plant extracts. The possible end result of this innovation is the production of anti-diabetic drugs of plant origin, which may still be expensive and out of reach of a good population of diabetics. Extracts are also found to have severe side effects. But most medicinal plants are vegetables which have been used in the preparation of diets man has lived on over the years; therefore preparing such medicinal plant as diet may be well tolerated and devoid of side effects. Reports are not available on the prophylactic or therapeutic efficacy of such dietary preparations, which appear to present a household, available and accessible prophylactic and therapeutic options for diseases in Africa since most household eat such vegetable in diets. This study assessed the effect of consumption of diets containing *Vernonia amygdalina* on haematological and immunological parameters of Streptozotocin induced experimental diabetic Wistar rats, with the view to evaluating its potential role in the management of haematological and immunological complications common among diabetics.

Vernonia amygdalina Del and several other species of *vernonia* have been used in native culture as folklore remedies for a variety of human ailments. All parts of the plants: roots, seeds, leaves, flowers, etc have been reported to possess curative properties [5]. In folklore medicine, *Vernonia amygdalina* is used as antihelmentic, antimalarial, laxative and fertility inducer [6,7]. It is also used to treat fever, hiccups, kidney problems and stomach discomforts [8]. Water extract is taken as blood tonic and when applied on wounds, enhances blood clothing. It is also used as insecticide and in the treatment of eczema. Among the people of sub-Saharan Africa, *Vernonia amygdalina* has more than 25 known medicinal uses of which about half are for treatment of intestinal ailment and parasite [9]. The antiparasitic effect of *Vernonia amygdalina* and the chemical principle in the plant responsible for such action are known. The chemical and phytochemical compositions have been reported by many workers and revealed a wealth of bioactive components that endow the plant with relevant nutritional, medicinal and industrial values. Several stigmastane-type saponins namely Vernonioside A, B, A_1 A_2, A_3, B_1, B_3, A_4 and C were isolated and fractionated from the leaves. Three (3) flavones namely: luteolin, luteolin 7-O-

B-glucuronoside, and Lutedin 7-O-B-glucoside were also isolated from the leaves. These flavones are responsible for the anti-oxidant properties which were more potent than those of butylated hydroxytoluene (BHT) when compared at equal concentration [6]. The A series were responsible for the bitter taste. Diet amended with the A series and saponins when fed to mice significantly reduced body and liver weight, increased urinary and faecal output, enlarged the stomach and small intestine and altered plasma and liver cholesterol levels [6]. Sesquiterpenes and steroidal constituents of *Vernonia amygdalina* had antiplasmodal effects and were effective against *Plasmodium falciparum in vitro* [10]. Vernodaline, vernolide and vernomygdine isolates of *Vernonia amygdalina* have cytotoxic effects [7] and are active against human carcinoma narsopharynx cells. Vernodaline and vernolide isolates also elicited antihumoral activities in leukaemia cells. Some peptides (edotide) from the aqueous extract of *Vernonia amygdalina* are antitumorigenic [11]. A body of information is available on the biochemical, toxicological and pharmacological effect of administration of extract of *Vernonia amygdalina* in normal rats and feeding leaves of *Vernonia amygdalina* to normal rats. Crude sapanin fraction of *Vernonia amygdalina* extract has haemolytic effects [12]. Acute toxicity test in rats showed LD_{50} of 1265.22±56 mg/kg [13] to 500 mg/kg [14]. Administering the leaf extract to rats at the dose range of 50 to 100 mg/kg body weight, had no effect on the hepatic cells [14] but was capable of restoring the hepatotoxicity caused by carbon tetrachloride [15]. The potential for using the extract of *Vernonia amygdalina* in the management of obesity has also been highlighted. Igile [16] reported that feeding rats with dried leaves of *Vernonia amygdalina* resulted in a significant reduction in weight and reduction of feed intake. Ibrahim et al. [17] after feeding rats with *Vernonia amygdalina* leaves for 2 months showed that although body weight was reduced after 2 months of chronic feeding, histology of testes, liver and kidney remained normal. Atangwho et al. [18] also observed that aqueous extract of *Vernonia amygdalina* administered to normal rats for 21 days resulted in significant reduction in weight gain. Works on root extract are few. Okokon and Onah [19] reported that the root extract of *Vernonia amygdalina* provided protection against pentylenetetrazole (PTZ)-induced lethality; and possessed significant analgesic activity against acetic acid induced abdominal constriction. It also has a significant antipyretic activity hence, a CNS depressant action.

Vernonia amygdalina also has wide reputation in the folkloric treatment of diabetes mellitus. An ethno-botanical survey of plants used in the management of diabetes in South Western Nigeria, revealed that out of the 22 plants identified and documented, leaves and roots of *Vernonia amygdalina* were the ones more frequently used by traditional attendants (34% and 64% respectively) compared to other plants [20]. Scientific studies on the effect of *Vernonia amygdalina* in the management of diabetes mellitus have been reported by many authors. The extract of the leaves of the plant has both hypoglyceamic [21] and antihyperglycemic effect on alloxan-induced diabetic rats [22]. Akah et al. [22] showed that the extract had positive impact on the lipid fragment in blood of the diabetic animals. Nimenido-Uadia [23] reported that aqueous extract was significant in reducing fasting plasma glucose, serum lipid fractions and ketone bodies in alloxan-induced diabetic rats. The effect of the aqueous leaf extract on body weight and serum lipid management both in diabetic and non-diabetic rats was reported by Atangwho et al. [18] and Ekaidem et al. [24]. The leaf extract showed antihyperlipidemic and hypolipidemic effects. The effect of the leaf extract of *Vernonia amygdalina* in the management of macrovascular complications was compared to those of *Caranthus roseus* and chlorpropamide [25] Furthermore, the ability of the leaf extract of *Vernonia amygdalina* to protect against internal tissue damage due to hyperglycaemia has been widely investigated. Extracts from the leaves of *Vernonia amygdalina* were shown to protect the hepatocyte [26] and Kidney [27] against hyperglycaemia induced damaged. Histology of the tissues corroborated results of biochemical indices. Nwanjo [28] in his studies demonstrated the hepatocyte protective effect of the leaf extract of *Vernonia amygdalina* using malondialdehyde as a marker. Also, the effect of dosage of the extract of *Vernonia amygdalina* in the management of diabetes mellitus has also been studied [24]. Ekiadem et al. [24] reported a non-dose dependent effect on blood glucose level but a dose dependent effect on HDL-cholesterol level. At higher dose, Atangwho et al. [18] demonstrated that the leaf extracts tend to precipitate hyponatremia (dilutional). Furthermore, the antidiabetic efficacy of a combination of extract of *Vernonia amygdalina* and those of *Azadirachta indica* (polyherbal therapy) has been demonstrated by

Ebong et al. [29]. In the study, it was shown that extracts from the two plants when combined produced a better gylcemic control and protection of the tissues, particularly the liver against damage compared to the monotherapy.

So far work done on *Vernonia amygdalina* in the management of diabetes have concentrated on the use of leaves and roots extracts. There are no reports on the role of dietary intake of the leaves. This research is significant because it could help diabetics, physicians and nutritionists to improve clinical outcome and quality of life.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Materials

Fresh but matured leaves of *Vernonia amygdalina Del* were collected from the Endocrine Research Farm, University of Calabar, and from University of Calabar Staff Village, Calabar in March, 2011. These leaves were authenticated by a Taxonomist and Voucher Specimens were deposited in the herbarium in the Department of Botany, University of Calabar. The leaves were selected to remove extraneous materials, washed and rinsed with distilled water and dried under shade. Dried leaves were milled using commercial feed mill machine (Artec model 40) to powder and sieved with 1 mm mesh to obtain fine leaf powder. Fine leaf powder were packaged in a well – labeled amber container and stored in the refrigerator at temperature 2-8°C until used for the preparation of rat chow. A portion of the sample was used for proximate composition.

2.2 Formulation of Experimental Diets

Feed ingredients include: leaf powder, soybean meal, maize meal, Garri, mineral premix, vitamin premix, L-lysine L-methionine and corn oil. These feed ingredients were purchased from Victory Livestock ltd, an accredited Livestock feeds/ vaccines/drug dealers, located at 79, Aka road, Uyo, Akwa Ibom State. Standard rat chows (growers) were formulated according to the nutritional reguirement of rat [30] (Table 1). Three (3) different diets were formulated namely: Control, VA-5%; VA-7.5%. Control diet differed from the other two diets because it did not contain leaf powder, but had all the other feed ingredients contained in the other diets. The other two diets contained leaf powder at five (5%) and seven and a half (7.5%) percent respectively. The percentage composition and nutrient analysis of the experimental diets are shown below Table 1.

2.3 Animals

Fifty (50) albino rats of Wistar strain (female only) weighing between 83-121 g were purchased from the animal house of the Faculty of Basic Medical Science, University of Uyo, Uyo, and transported in well ventilated cages to the animal house of the Department of Biochemistry,

Table 1. Percentage composition of experimental diets

Feed ingredient	Diets			
	Control	**Control + insulin**	**VA- 5%**	**VA- 7.5%**
Soybean meal(%)	33.78	33.78	31.03	30.53
Garri(%)	26	26	25	25
Maize meal(%)	38	38	37	35
L-Lysine(%)	0.18	0.18	0.18	0.18
L- Methionine(%)	0.17	0.17	0.17	0.17
Min/ vitamin(%)	0.25	0.25	0.25	0.25
DCP(%)	2.00	2.00	2.00	2.00
Bone meal(%)	1.00	1.00	1.00	1.00
Corn oil(%)	0.25	0.25	0.25	0.25
V. amygdalina (%)	-	-	5	7.5
Nutrient analysis				
CP(%)	18.40	18.40	18.31	18.47
CFAT(%)	4.30	4.30	4.01	3.97
CFIBRE(%)	3.71	3.71	4.27	4.58
ME(kcal/kg)	3219	3219	3214	3213

***Composition of premix:(n**utrient in Amount in 2.5kg)Vit A(I.U) 12,000,000,vit $D_{3\,(\,I.U)}$ 2,500,000, Vit E(mg) 20,000,vit $K_{3(}$ mg) 2,000,vit B1(mg) 2,000,vit B1 (mg) 5,000,Vit B6(mg) 4,000,vit B12(mg) 15,niacin(mg0 30,000,Pantotheic acid (mg) 11,000,Folic acid(mg) 1,500,Biotin(mg) 60,Choline chloride(mg)220,000,Antioxidant(mg) 1,250,Manganase (mg) 50,000, Zinc(mg) 40,000, Iron(mg) 20,000,Copper,(mg) 3,000,Iodine (mg) 1,000,Selenium (mg) 200,Cobalt(mg) 200*

University of Calabar, Cross River State, where they were kept throughout the duration of the experiment. The animals were allowed to acclimatize for two weeks. They were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature (25 ±5°C), relative humidity (50±5%) and twelve hour light/dark cycle. The animals were kept under the care of a trained animal technician and cared for according to Canadian Council on Animal Care: Guide to the care and use of experimental animals [31]. Animals were allowed free access to water and chow over a two weeks adaptation period.

2.4 Experimental Design and Induction of Experimental Diabetes Mellitus

The design consisted of fifty (50) female rats divided into 4 groups of diabetic and 1 groups of normal rats with 10 animals in each group. The rats in the diabetic groups were subjected to an overnight fast (12 hrs) prior to induction of diabetes. The weight of individual rats were measured and noted. Diabetes mellitus was induced in the diabetic groups by intraperitoneal injection of 55mg/kg body weight of Streptozotocin, (STZ) (sigma St. Louis, MO. USA) reconstituted in 0.1% M sodium citrate buffer. The pH of the buffer was adjusted to 4.5. Rats whose fasting blood glucose concentration were higher or equal to 200 mg/dl three days after the induction were confirmed diabetic and recruited in the study. Blood glucose concentration was determined using one touch Glucometer (Lifescan, Inc. 1995, Milpas, Galifornia, U.S.A) and Random assay kit (GDO-PAPmethod) based on Barham and Trinder, 1972) with blood obtained from the tail vein of the rats. Group 1 (normal control, NC) was fed with control diet; Group 2 (diabetic control, DC) was fed with control diet; Group 3 (diabetic treated with 5%,VA, 5%,VA) was fed with 5% *Vernonia* amygdalina(VA) diet; Group 4 (diabetic treated with 7.5%, VA, 7.5%, VA) was fed with 7.5% *Venonia amygdalina (VA*) diet; Group 5 (diabetic treated with insulin, INSULIN) was fed with control diet and treated with insulin, a standard therapeutic agent, which was introduced for comparison. Insulin dose used was 5 U/kg body weight (b.w), given subcutaneously (s.c) according to [32]. It was given once per day at 4.00 pm. Treatment lasted for 28 days.

2.5 Collection of Sample for Analysis

At the end of the 28 days, food and water were withdrawn. The rats fasted overnight. They were then euthanized under chloroform vapor and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles. The blood was emptied into EDTA sample bottles. The samples were used for analysis within 12 h of collection.

2.6 Determination of Full Blood Count (FBC) Using Automated Hematology Analyzer, KX2IN (non-cyanide hemoglobin analysis method)

Full blood counts including PCV (HCT), HB, RBC, WBC, platelet count, and differential WBC (lymphocytes and mixed), were estimated using the Sysmex® Automated Analyzer KX-2IN, Sysmex Corporation, Kobe-Japan.

2.7 CD_4^+ Count

The CD_4^+ lymphocyte was estimated by flow cytometry [33] using the cyflow automated cell counter (Parlec, Germany). Ten microlitres of CD_4^+ PE antibody was mixed with 5 ml of EDTA anticoagulated whole blood in a test tube. The mixture was incubated in the dark chamber for 15 min at room temperature of 22 -28°C. During incubation, the content of the tube was mixed every five min, eight hundred microlitres of buffer was added, mixed and plugged into the counter. After, counting the CD_4^+ cells, monocytes and noise were separated gated and the result was recorded.

2.8 Statistical analysis

The results were analyzed for statistical significance by one-way ANOVA using the SPSS statistical program and least square test (LSD) between group using MS excel programme. All data were expressed as mean ± SEM. P value <0.05 was considered significant.

3. RESULTS AND DISCUSSION

The effects of consumption of diet containing *Vernonia amygdalina* on some haematological and immunological parameters of diabetic rats are shown in Tables 2 and 3 respectively. The results in Tables 2 and 3 showed that the diabetic untreated rats (diabetic control) had significantly ($P< 0.05$) higher level of WBC (10.60±2.00 x 10^3 /µl) relative to the normal

control (6.87±2.28 x 10^3 /µl). The diabetic treated rats had white blood cells count (8.43±0.24 x 10^3 /µl for 5% VAD, 6.55±1.27 x 10^3 /µl for 7.5% VAD, and 7.43±0.33 x 10^3 /µl for Insulin treated group) that were significantly lower (P< 0.05) than the diabetic control (untreated) rats. The white blood cell count for the diabetic treated rats were not significantly different compared to the normal control except for rats that consumed 5%VA. RBC, HCT and HGB of the diabetic control were all significantly lower (P< 0.05) compared to the normal control, but the Platelet of the diabetic control was significantly higher compared to the normal control. The neutrophil, and CD_4^+ cell count (Table 3) were significantly (P< 0.05) higher for the diabetic control (30.33±4.95, 30.02±0.77 x 10^3 /µl respectively) compared to the normal control (23.00±1.93, 15.70±0.36 x 10^3 /µl respectively). The lymphocyte cell count for the diabetic control rats was significantly lower (P<0.05) compared to the normal control rats.

Treatment with the diets and insulin significantly (P< 0.05) lower, the WBC, neutrophil, and the CD_4^+ cell count relative to the untreated rats (diabetic control). The haematological and immunological parameters in the diabetic rats treated either with diets or insulin were not significantly different (P >0.05) from those of the normal control group.

Alteration of immune system may be the cause/or associated with diabetes mellitus [34] . In Type 1 diabetes there is a breakdown in immune regulation that lead to expansion of autoreactive CD_4^+ and CD_8^+ T cells, autoantibody- producing B lymphocytes and activation of the innate immune system [34,35]. In type 2 diabetes, there is deterioration of immunity and inflammatory process is enhanced due to increased level of immunoglobulin [34]. Type 2 diabetes is also inked by coincident presentation and alterations in Toll- like Receptor (TLR)- dependent B cell cytokine production [36]. The adaptive as well as innate immunity are decline in Type 1 diabetes but to a less extent in Type 2 diabetes [37,38] which may herald the susceptibility of patients to life threatening pyogenic infection [38] and existence of immune complex disease [39]. There is significant decrease in serum IgM in Type 1and 2 diabetes and this is related to presence of occult chronic infectious disease [40]. Urinary excretion of Ig M is increased and this is associated with an increased risk for cardiovascular mortality and renal failure [41]. Haematological changes consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets [42]. This is presented by alteration in platelet count and activity, coagulopathy, fibronolytic aberration, haemorrhagic factors and changes in endothelial metabolism [43]. The underlying cause of the changes in the immunity and hematology in diabetes mellitus is mainly due to oxidative damage [43].

Table 2. Effect of dietary consumption of *Vernonia amygdalina* on hematology of diabetic rats

Treatment	WBC(10^3/UL)	RBC(10^6/UL)	HGB(gm/dl)	HCT(%)	PLT(10^3/UL)
NC	6.87±2.28[a]	7.01±0.13[a]	13.40±0.04[a]	45.13±0.34[a]	6.19±19.08[a]
DC	10.60±2.00[b]	6.55±0.25[b]	12.76±0.27[b]	41.83±0.89[b]	7.15±39.61[b]
5%VA	8.43±0.24 [a]	7.13±0.14[a]	14.30±0.27[a]	50.13±0.73[a]	5.61±76.53[b]
7.5%VA	6.55±1.27[a]	6.58±0.09[a]	13.10±0.13[a]	45.10±0.62[a]	6.80±13.19[a]
INSULIN	7.43±0.33[a]	6.48±0.33[b]	12.43±0.58[b]	41.73±1.45[b]	1.04±195.21[c]

Means within the same column with different superscript are significantly different (P<0.05)

DC VS NC,RBC P= .03; HGB P= .02; HCT P= .02; WBC P=.02; PLT=.04; 5%VA VS DC, WBC P= .02; RBC P= .03, HGB P= .0; HCT P= .0, PLT P=.80; 7.5%VA VS DC WBC P= 0.04; RBC P =..04; HGB P= .01; HCTP= .0; PLT P= .02

Table 3. Effect of consumption of diet containing *Vernonia amygdalina* on differential white blood and CD_4^+ cell counts of diabetic rats

Treatment	Neutrophil(%)	Esenophil(%)	Basophil(%)	Lymphocyte(%)	Monocyte(%)	$CD4^+$(cells/ul)
NC	23.00±1.93[a]	2.67±0.56[a]	0.00±0.00	53.67±2.19[a]	0.67±0.33	15.70±0.36
DC	30.33±4.95[b]	1.33±0.55[b]	0.00±0.00	30.03±4.21[b]	0.00±0.00	30.02±0.77
5%VA	23.66±0.91[a]	3.00±0.73[a]	0.00±0.00	73.33±1.64[a]	0.00±0.00	0.00 ±0.00
7.5%VA	29.00±1.34[a]	3.50±1.11[a]	0.00±0.00	67.50±0.22[a]	0.00±0.00	18.95±0.42[a]
INSULIN	26.00±5.17[a]	5.00±0.73[d]	0.00±0.00	59.00±0.22[a]	0.00±0.00	17.87±0.51[a]

Means within the same column with different superscript are significantly different (P<0.05)

DC VS NC NEU P= .03, ESE P= .01; LYM P= .0; 5%VA VS DC NEU P= .02; ESE P= .04; LYM P =.0; 7.5% VAVS DC NEU P= .03; ESE P= 04, LYM P= .0

In this study, diabetic rats were observed to have alterations in hemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) count. The significant (P<0.05) reduction in the hemoglobin, and RBC cell and an increase in platelet and $CD4^+$ cells and the differential white blood cells count, neutrophil, lymphocyte, of the diabetic untreated rats (diabetic control) compared to the normal control and the diabetic treated rats may be due to the diabetic condition. As it pertains to haematology of the diabetic, terms such as anaemia in diabetes, atherosclerosis resulting from increased platelet aggregation, glycosylation of hemoglobin and of recent, even white blood cells have been discussed extensively [44]. Tables 2 and 3 showed that there was a significant increase in the White blood cell count of the diabetic control rats compared to the normal control and the diabetic treated rats. This may also be a manifestation of the diabetic condition. This is in line with normal physiologic response following the perception of an insult by the body. It is likely therefore that the damage caused by diabetes had caused the insult that contributed to the observed increase in WBC count. This is in agreement with Finlayson et al. [45] who reported that leucocytosis may occur in hepatic damage. Tables 2 and 3 also showed that the untreated diabetic rats had significant increase in platelets, lymphocyte and CD_4^+ counts compared to the normal control and treated diabetics. The increased immune cell counts may be the manifestations of the low grade inflammatory reactions associated with the atherosclerotic complications of diabetes mellitus [46]. Significant increase in platelet counts-thrombocytosis may follow haemorrhage, surgery or fracture of bones. Platelets have been prominently and critically implicated in the onset and pathogenesis of cardiovascular diseases (CVD) either of diabetics or of other causes. In diabetes for instance, the systems that maintain the integrity and patency of the vasculature including platelet and endothelial function, coagulation and fibrinolysis is impaired [46], shifting the balance in normal homeostasis in favour of thrombosis, hence increasing cardiovascular (CV) risk. The entire ischemic events usually begin with platelet activation-binding of platelet surface receptors to collagen, thrombin or components of atheromatous plague, to trigger hydrolysis of membrane phospholipids, mobilization of intracellular calcium and phosphorylation of some proteins [47]. Platelet function has been identified to be altered in several ways in diabetes including an increased release of TXA_2, accelerated platelet turnover, and an increased platelet aggregation [48]. This alteration in function is referred to as thrombocytopathy characterized by increased aggregability and adhesiveness [47]. Platelets from diabetics contain reduced antioxidant levels, which tends to be associated with increased aggregability and low platelet Vitamin C levels [49]. Addition of vitamin E has been shown to reduced or reversed platelet dysfunction [50]. Patients with type 1 and type 2 diabetes have also been reported to have increased population of platelet to express activation dependent adhesion molecules [51] A necessary parallel is also elevated serum levels of fibrinogen in most diabetics. Coagulation derangements and platelet function alteration are therefore strongly associated with diabetic and even pre-diabetic. Table 3 also shows that untreated diabetic rats had expanded autoreactive $CD4^+$ cells. The immunity was deteriorated with possible enhancement in inflammatory process [34].

Consumption of diet containing *Vernonia amygdalina* resulted in an increase in haemoglobin, red blood and lymphocyte cell count and a significant reduction of white blood cell count, circulating neutrophils, and CD_4^+ cells count. This perhaps helps to improve health condition of the diabetic rats. This is in line with the findings of Eteng et al. [25] who reported the reversal of anaemia in cadmium toxicity after the supplementation of diet, and Saliu et al. [43] who also reported reversal of anaemia in diabetic rats treated with some leafy vegetables. Although the mechanism of this effect is not well known, it is believed that the leaf antioxidant phytochemicals, vitamins and minerals [52] such as luteolin, luteolin 7-O-B-glucuronoside, Lutedin 7-O-B-glucoside, vitamin C, E, copper, selenium, chromium etc., might have help to reduce oxidative stress by mopping up free radicals in the diabetic rats. Although almost all organisms possess antioxidant defense and repair systems that have evolve to protect them, these system are insufficient to protect them completely in many disease conditions. This is why green leafy vegetables have been widely investigated for their protective action in diabetes mellitus since they contain valuable antioxidant, especially antioxidant vitamins including ascorbic acid, α-tocopherol, β- carotene and phenolics [53,54]. Asteraceae including *Vernonia amygdalina* and *Vernonia cinerea* [55] are rich in antioxidants phytochemicals. The iron and vitamins present in the leaf [52] might have help to replace those lost due to urinary excretion and this might have promoted the formation of RBC and

haemoglobin. Red blood cell counts can be a factor in erythropoietin process [56]. Increase in red blood cell count following consumption of vegetable diets might have resulted in an increased rate of erythropoietin production in diet treated group against the diabetic control. Increased circulating erythropoietin concentrations [56] might be able to elicit and enhance the production and expression of red cells antioxidant [57]. The decrease in circulating white blood cells might have been caused by the improvement in the health condition of the diabetic rats that withdrew some challenge. The same could be said for neutroplil. They are indicators for response to infection. The increase in the lymphocyte count in rat placed on *Vernonia* diets may be an indication of immunostimulation. The reduction in CD_4+ cell counts might indicate inhibition of immune cells recruitment in inflammatory vascular reactions. Similar anti-inflammatory activities have been reported for *Azardiracta indica* (neem) leaf extract [58,59]. The extract effect was attributed to inhibition of immune cells migration and phagocytosis, particularly for macrophage and neutrophils in respect to inflammatory stimuli. The extract was also seen to inhibit the induction of inducible nitric oxide synthase, prostaglandins E_2 (PGE_2) and interleukin 1 (IL-I) productions [57], thus controlling the increased vascular permeability associated with inflammatory reactions.

4. CONCLUSION

In conclusion, consumption of diet containing *Vernonia amygdalina* leaves have been shown to reverse diabetic associated disturbances of immunological and haematological parameters of the experimental rats and may signify positive effect of the diets on immunological and haematopoietic system of the experimental rats and this may be associated with its antioxidant potentials. Therefore, consumption of diets containing *Vernonia amygdalina* may play a significant role in the management of haematological and immunological complications common among diabetics. This study is recommended for further studies.

CONSENT

Not applicable.

ETHICAL APPROVAL

College Ethical committee approval.(reference number: uc/pgp/bmec/11/1/01).

ACKNOWLEDGEMENTS

The authors wish to acknowledge Prof Patrick Ebong, University of Calabar and the financial assistance given by the Education Trust Fund, Federal Government of Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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