British Journal of Applied Science & Technology
6(5): 534-544, 2015, Article no.BJAST.2015.108
ISSN: 2231-0843

SCIENCEDOMAIN *international*
www.sciencedomain.org

Modulation of Immunological and Hematological Disturbances of Diabetes Mellitus by Diets Containing Combined Leaves of *Vernonia amygdalina* and *Gongronema latifolium*

Henry D. Akpan[1*] and Itemobong Ekaidem[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria,
[2]Department of Chemical Pathology, Faculty of Clinical Sciences, University of Uyo, Uyo, Nigeria.

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 IE 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.

Article Information

DOI: 10.9734/BJAST/2015/14266
Editor(s):
(1) Selvakumar Subbian, Laboratory of Mycobacterial Pathogenesis and Immunity, Public Health Research Institute (PHRI) at Rutgers Biomedical and Health Sciences, Newark, USA.
Reviewers:
(1) Anonymous, KSA.
(2) Anonymous, Turkey.
(3) Anonymous, Poland.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=768&id=5&aid=7666

Original Research Article

Received 25th September 2014
Accepted 12th December 2014
Published 7th January 2015

ABSTRACT

Background: Immunological and hematological alterations often occur in diabetes mellitus as a result of oxidative stress. This study investigated the effects of consumption of diets containing *Vernonia amygdalina* leaves on some markers of immunology and hematology in Streptozotocin - induced diabetic wistar rats with the view to evaluating its involvement in the management of immunological and hematological complications among diabetics.
Methods: The design consisted of eighty rats randomly divided into eight groups of 10 rats per group. Groups 2, 3, 4, 5, 6, 7 and 8 were diabetic, while group 1 was normal control. Groups 1 and 2 were fed with control diet. Group 3 to 7 were fed with diets containing *Vernonia amygdalina, or Gongronema latifolium* or combined leaves of *Vernonia amygdalina* and *Gongronema latifolium*. Group 8 was fed with the control diet but treated with Insulin. Feed and water were given *ad-libitum* for 28 days.

**Corresponding author: E-mail: henry_dan4u@yahoo.com;*

Results: Results showed that diabetic rats consuming diets of *Vernonia amygdalina*, *Gongronema latifolium* leaves or the combined leaves diets all had significant (P<0.05) increase in the red blood cell (RBC), hemoglobin, and lymphocyte counts relative to the diabetic control. All the diets resulted in significant (P<0.05) reduction in the level of white blood cell (WBC), platelets, neutrophil, and CD_4^+ cell count relative to the diabetic control. The results for *Vernonia amygdalina* and *Gongronema latifolium* diets were similar to insulin on the measured parameters and their levels were not significantly different (P<0.05) when compared to the normal control. Diets containing combined leaves of *Vernonia amygdalina* and *Gongronema latifolium* performed better than insulin and the measured parameters were numerically equals to the normal control.

Conclusion: The findings of the present study indicate that consumption of diets containing *Vernonia amygdalina* or *Gongronema latifolium* leaves or in combination at the levels in this study have significant role in ameliorating hematological and immunological disturbances associated with diabetes mellitus. The combined leaf diet performed better due to positive synergy among the leaves bioactive agents. Consumption of diets containing *Vernonia amygdalina* or *Gongronema latifolium* leaves or in combination may be recommended as dietary therapies for management of immunological and hematological complications in diabetes mellitus.

Keywords: Diabetes; diet; Vernonia amygdalina; Gongronema latifolium; immunological; hematological; cardiovascular risk.

ABBREVIATIONS

WBC (White Blood Cells); RBC(Red Blood Cells); HGB (Hemoglobin); HCT (Hematocrit); MCV (Mean Cell Volume); MCH (Mean Cell Hemoglobin); MCHC (Mean Corpuscular Hemoglobin Concentration); NC (Normal Control); DC (Diadetic Control); INSD (Insulin Treated); GL (Gongronema Latifolium); VA (Vernonia Amygdalina).

1. INTRODUCTION

Diabetes mellitus (DM) is a major global health problem [1,2]. According to estimates of the World Health Organisation, the number of people suffering from diabetes worldwide is increasing at an alarming rate. There were 346 million people suffering from DM 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]. Cure eludes physicians and many sufferers especially in poor countries of the world cannot meet the cost of conventional drugs. Therefore, it has become desirable to investigate alternative sources of medicament.

Many reports have been made in recent years regarding the relationship between DM and dietary pattern [5]. Many of these studies have reported that vegetable intake reduces the onset of DM and improves plasma glucose control, blood lipids and renal function in diabetic patients thus reducing the risk of cardiovascular events [5]. There are few studies on vegetarian diets in diabetes at present, considering the enormous number of vegetables and plant products in the world. Therefore, novel plants and plant components that may have significant outcome and provide an alternative to drug therapy are desirable. There is scarcity of literature on the effect of combining different plants leaves or other components in the same diet for DM management. This study assessed the effect of consumption of diets containing combined leaves of *Vernonia amygdalina* and *Gongronema latifolium* on hematological and immunological parameters of Streptozotocin- induced experimental diabetic Wistar rats so as to evaluate the role of the diets in the management of hematological and immunological complications common among diabetics.

The underlying cause of the disturbances in the immunity and hematology in diabetes mellitus is mainly due to oxidative damage [6]. Although almost all organisms possess antioxidant defense and repair systems that have evolve to protect them, these system are insufficient to protect completely in many disease conditions. For this reason, green leafy vegetables have been widely investigated for their protective action in DM since, they contain valuable antioxidant, especially antioxidant vitamins

including ascorbic acid, α- tocopherol, β-carotene and phenolics [7,8].

Vernonia amygdalina Del popularly called bitter leaf is a species of Vernonia that belong to the family compositae (Asteraceace). *Congronema latifolium* Benth is of the family (Asclepiadaceaae). It is a tropical rainforest plant, a climber with tuberous base limited in distribution to wet and dry forest of tropical Africa [9,10], Guinea Bissau and western Camerouns [11]. These plants are popularly used in the preparation of soups in many African countries.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Materials

Fresh but matured leaves of *Vernonia amygdalina* and *Gongronema latifolium* 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 until the leaves were dried. Dried leaves of *Vernonia amygdalina* and *Gongronema latifolium* were milled separately using commercial feed mill machine (Artec model 40) to powder and sieved with 1mm mesh to obtain fine leaf powder. Fine leaf powder were packaged separately in a well- labeled amber container and stored in the refrigerator at temperature 2-8°C until used for the preparation of rat chow.

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 requirement of rat [12] (Table 1). Seven (7) different diets were formulated namely: Control, VA-5%; GL-5%, VA+GL-5%, VA-7.5%, GL-7.5%, VA+GL-7.5%. The control diet differed from the other five diets because it did not contain leaf powder, but had all the other feed ingredients contained in the other diets. The other five diets contained leaf powder at five (5%) or seven and a half (7.5%) percent. The percentage composition and nutrient analysis of the experimental diets are shown Table 1.

2.3 Animals

Eighty (80) 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, University of Calabar, Calabar, 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. Approval was granted by the Ethics committee of the College of Basic Medical Science, University of Calabar and the animals were kept under the care of a trained animal technician and cared for according to [13]. Animals were allowed free access to water and chow over a two weeks adaptation period and closely monitored.

2.4 Experimental Design and Induction of Experimental Diabetes Mellitus

The design consisted of eighty (80) female rats divided into 7 groups of diabetic and 1 group of normal rats with 10 animals in each group. Diabetic rats were obtained by subjecting some rats to an overnight fast (12 hrs) prior to induction of DM. The weight of individual rats were measured and noted. DM was induced by intraperitoneal injection (once) of 55 mg/kg body weight of Streptozotocin (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) with blood obtained from the tail vein of the rats. The seven diabetic groups were randomly assigned to the seven experimental diets: Control, VA-5%, GL-5%, VA+GL-5%, VA-7.5%, GL-7.5% and VA+GL-7.5 %. Insulin, a standard therapeutic agent was introduced for comparison. Insulin dose used

was 5 U/kg body weight (b.w), given subcutaneously (s.c) according to [14]. It was given once per day at 4.00 pm. That is, the group that received Insulin was fed with the control diet. Rats in the normal group were fed with the control diet. Feed and water (Tap water) were given *ad-libitum*. Treatment lasted for 28 days. Daily recording of feed intake, weight of animals and blood glucose concentration were kept.

2.5 Collection of Sample for Analysis

At the end of the 28 days, food and water were withdrawn. The rats were allowed to fast 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 12h of collection.

2.6 Determination of Full Blood Count (FBC) Using Automated Hematology Analyzer, KX2IN (Non-cyanide Hemoglobin Analysis Method)

Full blood counts including packed cell volume (PCV), hemoglobin (HGB), RBC, WBC, platelet count, differential WBC (lymphocytes and mixed), red cell indices: Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) 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 [15] using the cyflow automated cell counter (Parlec, Germany). Ten microlitres of CD_4^+ PE antibody was mixed with 5ml 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. Student T tests were used to assess differences between individual variables. 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 combined leaves of *Vernonia amygdalina* and *Gongronema latifolium* on some hematological and immunological parameters of diabetic rats are shown in Tables 2 and 3 respectively. The results in Table 2 showed that the diabetic control had significantly higher (P<0.05) 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 rats treated with diets and Insulin had WBC count that were significantly lower (P<0.05) compared to the diabetic control. The effect of *Vernonia amygdalina* diet on WBC appeared to be dose dependent, better at the higher inclusion level (7.5%). *Gongronema latifolium* diet also had dose dependent effect on WBC, but better at lower level of inclusion (5%). Combination of *Vernonia amygdalina* and *Gongronema latifolium* leaves in the diet exerted an effect that brought the WBC to a level numerically equal to those of normal control and this treatment was better than Insulin particularly at 5% inclusion level. RBC, HGB and PCV as well as red blood cell function indices: MCV, MCH and MCHC 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 (P<0.05) compared to the normal control (Table 2). Treatment with diets and insulin significantly increased (P<0.05) the RBC, HGB and PCV as well as red blood cell function indices: MCV, MCH and MCHC of the diabetic treated rats (Table 2). The increased in the RBC, HGB and PCV as well as red blood cell function indices: MCV, MCH and MCHC following consumption of the diets were not dose dependent. However, the RBC, HGB and PCV tended to increase more at higher inclusion level of *Vernonia amygdalina* (7.5%) but at lower inclusion level of *Gongronema latifolium* (5%). The MCV, MCH and MCHC tended to increase more at lower level of inclusion of *Vernonia amygdalina.*

Table 3 showed that the diabetic control had neutrophil, basophil, monocyte and CD_4^+ cell count that were significantly higher (P<0.05) (30.33±4.95%, 1.81±0.00%, 5.40±0.00%, and 51.53±0.77 x 10^6/l, respectively) compared to the

normal control (23.00±1.93%, 0.00±0.00%, 4.67±0.33% and 15.70±0.36 x 10^6/l respectively). The esenophil and lymphocyte cell counts for the diabetic control rats (1.33±0.55% and 43.33±4.21 respectively) were significantly lower (P<0.05) compared to the normal control (2.67±0.56 and 70.67±2.19 respectively). Treatment with the diets and Insulin significantly lower (P<0.05) the neutrophil, monocyte and the CD_4^+ cell count but increased the esenophil, and lymphocyte cell count relative to the diabetic control. The *Vernonia amygdalina* diet had dose dependent effect on the neutrophil and was better at low inclusion level (5%). The effect of *Gongronema latifolium* diet on the neutrophil was not dose dependent but was better at the high inclusion level (7.5%). Combination of *Vernonia amygdalina* and *Gongronema latifolium* in the diet exerted better effect on the neutrophil than using *Vernonia amygdalina* or *Gongronema latifolium* alone. This brought the neutrophil to a level that was numerically equal to that of the normal control; and this treatment was better than Insulin particularly at 5% inclusion level. The *Vernonia amygdalina* diet had no dose dependent effect on esenophil and lymphocyte. The *Gongronema latifolium* diet had no dose dependent effect on esenophil, lymphocyte, monocyte and CD_4^+ cell count. Combination of *Vernonia amygdalina* and *Gongronema latifolium* in the diet exerted better effect on the esenophil, lymphocyte, monocyte and CD_4^+ cell count than using either *Vernonia amygdalina* or *Gongronema latifolium* alone. Their levels were numerically equal to those of the normal control. Treatment of DM using the combined leaves in the diet was better than using Insulin particularly at 5% inclusion level of the leaves.

Type 1 diabetes is characterized by overt destruction of pancreatic islet cells. Individual sufferers depend on insulin. Many patients with type 1diabetes fail to meet recommended glycemic goals regardless of the recognition of optimal glycemic control as a key component for improving clinical outcomes and quality of life [16]. Patients and physician related barriers to the adoption of insulin therapy include fear and anxiety about infecting insulin, concerns about side effects, the availability and high cost of insulin and personal health beliefs in regard to the use of insulin [16]. There is an unmet need for an alternative therapy that provides optimal glycemic control, well tolerated, and improves patient adherence. There is upsurge of interest in leafy vegetables in view of the abundance of natural bioactive agents. In this study, a model of Type 1 diabetes was developed in rats to investigate the effect of consumption of diets containing combined leaves of *Vernonia amygdalina* and *Gongronema latifolium* on hematological and immunological parameters and to compare with Insulin which is the standard therapy. The diabetic control rats were observed to have alterations in hemoglobin (HGB), red blood cell (RBC) and white blood cell (WBC) count. The significant (P<0.05) reduction in the HGB, and RBC cell and an increase in the WBC, neutrophil, lymphocyte, platelet and $CD4^+$ cells of the diabetic control compared to the normal control and the diabetic treated rats may be a manifestation of the diabetic condition. As it pertains to hematology of the diabetic, terms such as anaemia, atherosclerosis resulting from increased platelet aggregation, glycosylation of hemoglobin and of recent, white blood cells have been discussed extensively [17,18]. The normal physiologic response following the perception of an insult by the body is an increase in WBC count. It is likely that the damage caused by DM contributed to the observed increase in WBC count. This is in agreement with Finlayson [19] who reported that leucocytosis may occur in hepatic damage. White blood cell count is increased in obesity [20] and is a risk factor for atherosclerosis [21]. An elevated WBC count is present in impaired glucose tolerance (IGF) [21] and is associated with macro- and micro angiopathic complications in type 2 diabetes [22]. High WBC count has been shown to be associated with insulin resistance, decrease in insulin action [23] and is predictive of the development of type 2 diabetes [24,21], coronary artery disease [25], stroke [25] and diabetes micro- and macrovascular complications [22].

The RBC, HGB, PCV alongside with erythrocyte function indices: MCV, MCH and MCHC all reduced significantly (P<0.05) while platelet increased significantly (P<0.05) in the diabetic control compared to the normal control and treatment groups. The low HGB in diabetic control group may be associated with kidney damage due to DM. The decrease in MCV, MCH and MCHC values, observed in the diabetic control group is an indication of abnormal hemoglobin synthesis, failure of blood osmoregulation, and plasma osmolarity [26] that may be associated with DM. The increase in the level of platelet in the diabetic control group may be due to inflammatory responses caused by DM and correlate with the level and sustenance of high blood glucose level [27]. Platelets assume

an important role in signaling of the development of advanced atherosclerosis in DM [27].

We observed that consumption of all the diets and administration of Insulin significantly reduced (P<0.05) WBC and platelet but significantly increased (P<0.05) RBC, HGB, PCV, MCV, MCH and MCHC in the diabetic treated group compared to diabetic control. The results obtained for RBC suggest that the diets could stimulate the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce RBC [28]. This is supported by the improved level of MCH and MCHC [29]. Thus there was a restoration of oxygen carrying capacity of the blood in the diabetic treated group. Although the mechanism of this effect is not well known, it is assumed that the antioxidants in the leaves [30] might have help to reduce oxidative stress of DM and thus improve the metabolism and well being of the RBC. The leaves are rich in blood formatting minerals and vitamins [30] which might have help to replenish those lost due to urinary excretion and thus promoted the formation of RBC and HGB. RBC counts can be a factor in erythropoietin process [31], leading to an increased in concentration of circulating erythropoietin [31]. Increased circulating erythropoietin may be able to elicit and enhance the production and expression of red cells antioxidant [32] and enhance their ability to lower lipid peroxidation level [33] that causes hemolysis of erythrocytes. The result we obtain is in line with the findings of Eteng [34] who reported the reversal of anaemia in cadmium toxicity after the supplementation of diet, and Saliu [6] who also reported reversal of anaemia in diabetic rats treated with some leafy vegetables. Some parameters such as WBC and neutrophil depended on the dosage of the leaves in the diet. The mechanism is not known but may relate to the level of the bioactive agents found. At low dose level there might be inadequate amount of the bioactive agents. The combination of the two leaves help to synergies them so that bioactive agents that were not found in one of the leaves were compensated by the other. *Vernonia amygdalina* and *Gongronema latifolium* leaves contain varying levels of flavonoids, tannins, saponins, polyphenols, alkaloids, hydrocyanic acid, vitamin A, E, C, riboflavin, thiamin, niacin, iron, selenium, and chromium [30]. Table 3 shows that the neutrophil, basophil, monocyte and CD_4^+ *c*ell count were significantly increased (P<0.05) in the diabetic control group compared to the treatment group and the normal control, while the lymphocyte was significantly reduced (P<0.05) in the diabetic control group compared to the treatment group and the normal control. Polymorphonuclear cells, including monocytes as well as lymphocytes make up the peripheral blood leukocytes. In a state of hyperglycemia, polymorpho- and mononuclear leukocytes can be activated by advanced glycation end products [35], oxidative stress [36], angiotensin II [37], and cytokines [38]. The release of cytokines, such as TNF-α [39], transforming growth factor-1 [40], superoxide [41], nuclear factor κB (NF-κB) [42], monocyte chemoattractant protein 1, interleukin-1β, and others [39], may activate leukocytes to participate in the pathogenesis of diabetic micro- and macrovascular complications. Studies showed that DM in mice was accompanied by moderate neutrophilic leukocytosis and prolonged circulation times of neutrophils and monocytes, and a shortened circulation time of lymphocytes, which increases the susceptibility to infection [43]. The raised leukocyte count reflected low-grade inflammation [43]. The mechanism responsible for leukocytosis in DM is largely unknown but recent evidence shed light on how elevated WBC increase cardiovascular and stroke risk linking inflammation mediated by neutrophil to insulin resistance [44]. We suggest that the raised leukocyte count may be inflammatory response to DM. The decrease in the WBC count, in diabetic treated rats shows the anti-inflammatory property of the *Vernonia amygdalina, Congronema latifolium* and the combined leaf diets. Lymphocytes and CD_4^+ cells are the effectors cells of the immune system we studied. The increase in the lymphocyte count in rats placed on *Vernonia amygdalina, Congronema latifolium* and combined leaf diets may be an indication of immunostimulation. The reduction in CD_4^+ cell counts might indicate reduced stimulation of immune cells recruitment in inflammatory reactions. Similar anti-inflammatory activities have been reported for *Azardiracta indica* (neem) leaf extract [45,46]. 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 [45], thus controlling the increased vascular permeability associated with inflammatory reactions.

Table 1. Percentage composition and nutrient analysis of diets

Feed ingredients	Diets:						
	Control	**VA-5%**	**GL-5%**	**VA+GL-5%**	**VA-7.5%**	**GL-7.5%**	**VA+GL-7.5%**
Soybean meal (%)	33.78	31.03	31.03	31.03	30.53	30.53	30.53
Garri (%)	26	25	25	25	25	25	25
Maize meal (%)	38	37	37	37	35	35	35
L-Lysine (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Methionine (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Min/vitamin (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DCP (%)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Corn oil (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
V. amygdalina (%)	-	5	-	2.5	7.5	-	3.75
G. latifolium (%)	-	-	5	2.5	-	7.5	3.75
Analysis:							
CP	18.40	18.31	18.40	18.34	18.47	18.46	18.48
CFAT	4.30	4.01	4.14	4.07	3.97	4.16	4.06
CFIBRE	3.71	4.27	4.15	4.21	4.58	4.41	4.49
ME	3219	3214	3218	3216	3213	3218	3216

Composition of premix:*(nutrient 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.* ***Manufactured by Megabiotics Nigeria LTD***

Table 2. Effect of consumption of diet containing combined leaves of *Vernonia amygdalina* and *Gongronema latifolium* on hematology of diabetic rats

Treatment	WBC (10^3/UL)	RBC (10^6/UL)	HGB (gm/dl)	HCT (%)	PLT (10^3/UL	MCV (fl)	MCH (pg)	MCHC (g/dl)
NC(normal control)	6.87±2.28[a]	7.01±0.13[a]	15.40±0.04[a]	45.13±0.34[a]	6.19±19.08[a]	72.75±1.02[a]	25.11±0.68[a]	35.20±5.32[a]
DC(diabetic control)	10.60±2.00[b]	6.13 ±0.14[b]	12.30±0.27[b]	30.13±0.73[b]	7.15±39.61[b]	50.15±3.20[b]	16.25±3.78[b]	29.20±2.05[b]
5%VA	8.43±0.24 [c]	6.55±0.25[a]	12.76±0.27[a]	41.83±0.89[a]	5.61±76.53[a]	72.82±1.00[a]	26.65±1.00[a]	32.00±2.01[a]
5%GL	7.20±1.26[a]	7.00±0.22[a]	18.23±0.26[a]	45.13±1.44[a]	6.77±58.08[a]	70.30±0.28[a]	22.13±0.32[a]	35.34±1.25[a]
5%VA+GL	6.87±3.10[a]	6.91±0.15[a]	15.89±1.02[a]	45.00 ±0.04[a]	5.59±1.72[a]	73.84±0.01[a]	20.13±0.02[a]	36.00±2.25[a]
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]	70.25±0.10[a]	26.00±1.40[a]	30.21±1.00[a]
7.5%GL	8.33±0.44[c]	7.64±0.18[a]	18.40±0.43[a]	46.83±1.38[a]	6.28±60.32[a]	71.25±0.50[a]	26.17±0.50[a]	34.21±3.41[a]
7.5%VA+GL	7.21±2.21[a]	6.23±2.39[a]	13.00±0.11[a]	44.65±0.01[a]	6.11±3.21[a]	70.00±4.21[a]	26.00±2.32[a]	31.76±0.00[a]
INSD insulin treated)	7.43±0.33[a]	6.48±0.33[a]	12.83±0.58[a]	41.73±1.45[a]	6.04±18.21[a]	67.28±0.28[a]	20.05±1.26[a]	30.11±1.25[b]

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

Table 3. Effect of consumption of diet containing combined leaves of *Vernonia amygdalina* and *Gongronema latifolium* on differential white blood and CD_4^+ cells count of diabetic rats

Treatment	Neutrophil (%)	Esenophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)	$CD4^+$cell count (10^6/L
NC(normal control)	23.00±1.93[a]	2.67 ±0.56[a]	0.00±0.00	70.67±2.19[a]	4.67±0.33[a]	15.70±0.36[a]
DC(diabetic control)	30.33±4.95[b]	1.33±0.55[b]	1.81±0.00	43.33±4.21[b]	5.40±0.00[b]	51.53±0.77[b]
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
5%GL	24.33±4.07[a]	1.66±0.76[a]	0.00±0.00	58.66±14.15[a]	4.00±0.00[a]	19.29±0.47[a]
5%VA+ GL	22.95±1.06[a]	2.78±1.43[a]	0.00±0.00	70.77±2.30[a]	4.00±1.21[a]	13.33±3.42[a]
7.5%VA	29.00±1.34[b]	3.50±1.11[a]	0.00±0.0	67.50±0.22[a]	0.00±0.00	18.95±0.42[a]
7.5%GL	22.00±2.22[a]	1.66±0.21[a]	0.00±0.00	61.33±2.43[a]	4.00±0.00[a]	20.25±0.77[a]
7.5%VA+ GL	23.01±1.21[a]	1.98±1.00[a]	0.00±0.00	64.76±1.32[a]	4.00±0.00[a]	19.00±0.10[a]
INSD(insulin treated)	26.00±5.17[b]	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)

4. CONCLUSION

The findings of the present study indicate that consumption of diets containing *Vernonia amygdalina* or *Gongronema latifolium* leaves or combination have significant role in ameliorating hematological and immunological disturbances associated with DM. The combined leaf diet performed better due to positive synergy among the leaves bioactive agents. The mechanism may be related to the prevention or inhibition of lipid peroxidative system by the leaves antioxidants, maintenance of cellular integrity, and attenuation of pro-inflammatory cytokine production. Consumption of diet containing combined leaves of *Vernonia am*ygdalina and *Congronema latifolium* at 5% was better than using Insulin and may be recommended as a dietary therapy for management of immunological and hematological complications in DM.

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.

REFERENCES

1. Mahsud MAJ, Khan A, Hussain J. Hematological Changes in Tobacco using Type 2 Diabetic Patients. Gomal J Med Sci. 2010;8:8-11.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: Prevalence, numerical estimates, and projections. Diabetes Care. 1998;21:1414-31.
3. World Health Organization; 2011. [Last accessed on 2011 Nov 17]. Available:http://www.who.int/mediacentre/factsheets/fs312/en/
4. World Health Organisation (WHO) (2008). Diabetes in the WHO country and regional data; 2008. Available:ww.who.int/diabetes/facts/wpworldfigures/en/index.html)
5. Keiko Takahashi, Chiemi Kamada, Hidenori Yoshimura, Ryota Okumura, Satosh Iimuro, Yasuo Ohashi, Atsushi Araki, Hiroyuki Umegaki, Takashi Sakurai, Yukio Yoshimura, Hideki Ito. Effects of total and green vegetable intakes on glycated hemoglobin A1c and triglycerides in elderly patients with type 2 diabetes mellitus: The Japanese Elderly Intervention Trial. Geriatrics & Gerontology International. 2012;(12):50–58l.
6. Saliu JA, Elekofehinti OO, Komolafe K, Oboh G. Effect of some green leafy vegetables on the hematological parameters of diabetes rat. J. Nat. Pro. Plant. Resour. 2012;2(4):482-485.
7. Chu YF, Sun X, Wu X, Liu R. H. Antioxidant and antiproliferative activities of vegetable. J. Agric Food Chem. 2002;50:6910- 6916.
8. Oboh G. JBT: Rocha, leading edge. Antioxid Research. 2007;35-64.
9. Ugochukwu NH, Babady NE. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life science. 2002;73:1925-1938.
10. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress indices in hepatocytes of diabetic rats. Journal of Biosciences. 2003;28:1-5.
11. Francis VU, Nelson CO, Simon JU. Influence of *Gongronema Latifolium* Leaf-Extracts Treatment on Some Hepatic Enzymes Activity in Rats. Journal of Natural Sciences Research. 2013;3(5)32-35.
12. National Research Council: Nutrition reguirement of laboratory animals, USA. 1995;38.
13. Canadian Council on Animal Care. Guide to the care and use of experimental animals. Ottawa, Ontario, Canada: CCAC. 1993;2.
14. Sonia B, Scrinivasan BP. Investigations into the antidiabetic activity of *Azardiracta indica*. Indian Journal of Pharmacology. 1999;31:138-141.
15. Center for Disease Control and Prevention: Revised guidelines for performing CD_4^+ T-cell determinations in persons infected with Human Immunodeficiency Virus (HIV). MMWR Recomm. Rep. 1997;46(RR-21):1-29.

16. Priscilla A. Hollander: Evolution of a Pulmonary Insulin Delivery System (*Exubera*) for Patients With Diabetes Med Gen Med. 2007;9(1):45-51.
17. Thomas M, Tsalamandris C, Maclssac R, Jerums G. Anemia in diabetes: An emerging complication of microvascular disease. Current Diabetes Reviews. 2005;1:107-126.
18. Colwell JA, Nesto RW. The platelets in diabetes: Focus on prevention of ischemic events. Diabetes Care. 2003;26:2181-2188.
19. Finlayson NDC, Hayes PC, Simpson KJ. Diseases of the liver and biliary system. In: Davidson's principles and practice of medicine. Eds. Haslet C, Chilvers ER, Hunker JAA. Boon NA, 18th ed. Churchill Living Sone, Edinburgh. 1999;683-736.
20. Kullo IJ, Hensrud DD, Allison TG. Comparison of numbers of circulating blood monocyte in men grouped by body mass index (< 25, 25 to <30, > or = 30. Am. J. Cardiol. 2002;89:1441-1443.
21. Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, Itara H, Kohno N.Elevated white blood cell count in subjects with impaired glucose tolerance. Diabetes Care. 2004;27:491-496.
22. Tong PC, Lee KF, So WY, Ng MH, Chan WB, Lo MK, Chan NN, Chan JC. White blood cell count is associated with macro and microvascular complications in Chinese patients with type 2 diabetes. Diabetes Care. 2004;27:216-22.
23. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA. High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. Diabetes. 2002;51(2):455-61.
24. Barbara GB, Małgorzata S, Joanna G, Beata K, Justyna S, Mieczysław W, Anhelli S. Changes in inflammatory biomarkers after successful lifestyle intervention in obese children. Endokrynologia Polska/ Polish Journal of Endocrinology. 2011; 62:499- 505.
25. Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and white men and women: Atherosclerosis risk in communities study. Am J Epidemiol. 2001;154:758-64.
26. Stookey JD, Burg M, Sellmeyer DE, Greenleaf JE, Arieff A, Van Hove L. A proposed method for assessing plasma hypertonicity in vivo. Eur J Clin Nutr. 2007;61:143-6.
27. Herzog CA, Mangrum JM, Passman R: Sudden cardiac death and dialysis patients. Semin Dial. 2008;21:300–307.
28. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev. 2012;9: CD004863.
29. Abu-Zaiton AS. Antidiabetic activity of *Ferula asafoetida* extract in normal and alloxan induced diabetic rats. Pak J Biol Sci . 2010;13:97-100.
30. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GC: Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica, Vernonia amygdalina and Gongronema latifolium*. African Journal of Biotechnology. 2009;8(18):4685-4689.
31. Inomata H, Itoh M, Imai H, Sato T. Serum erythropoietin levels as a novel marker of severity of diabetic nephropathy. Nephrolog. 1997;7:426-9.
32. Digicayliogu M, Lipton MA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak 2 and NF-kappa signalling cascades. Nature. 2001;412: 641-647.
33. Mahmoud AM. Influence of rutin on biochemical alterations in hyperammonemia in rats. Exp Toxicol Pathol. 2012;64:783-9.
34. Eteng MU, Onwuka FC, Akpanyung EO, Oguchukwu NC, Bassey SC, Nwankpa P. Reversal of cadmium induced toxicity following dietary supplementation with garlic, ginger and cabbage in male wistar rats. J. Nat. Prod. Plant Resour. 2012; 2(1):169-174.
35. Pertynska-Marczewska M, Kiriakidis S, Wait R, Beech J, Feldmann M, Paleolog EM. Advanced glycation end products upregulate angiogenic and pro-inflammatory cytokine production in human monocyte/ macrophages. Cytokine. 2004; 28:35-47.
36. Shurtz-Swirski R, Sela S, Herskovits AT, Shasha SM, Shapiro G, Nasser: Involvement of peripheral polymorphonuclear leukocytes in oxidative

stress and inflammation in type 2 diabetic patients. Diabetes Care. 2004;24:104-10.
37. Lee P, Peng H, Gelbart T, Beutler E: The IL-6- and lipopolysaccharide-induced transcription of hepcidin in HFE-, transferring receptor 2-, and beta 2-microglobulin deficient hepatocytes. Proc Natl Acad Sci. 2004;101:9263-5.
38. Scherberich JE. Proinflammatory blood monocytes: Main effector and target cells in systemic and renal disease; background and therapeutic implications. Int J Clin Pharmacol Ther. 2003;41:459-64.
39. Shanmugam N, Reddy MA, Guha M, Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. Diabetes. 2003;52:1256 64.
40. Korpinen E, Groop PH, Fagerudd JA, Teppo AM, Akerblom HK, Vaarala O. Increased secretion of TGF-beta1 by peripheral blood mononuclear cells from patients with type 1 diabetes mellitus with diabetic nephropathy. Diabet Med. 2001;18:121-5.
41. Kedziora-Kornatowska KZ. Production of superoxide and nitric oxide by granulocytes in non-insulin-dependent diabetic patients with and without diabetic nephropathy. IUBMB Life. 1999;48:359-62.
42. Hofmann MA, Schiekofer S, Kanitz M, Klevesath MS, Joswig M, Lee V: Insufficient glycemic control increases nuclear factor-kappa B binding activity in peripheral blood mononuclear cells isolated from patients with type 1 diabetes. Diabetes Care. 1998;21:1310-6.
43. Kozlov IA, Novitski VV, Baĭkov AN. Kinetics of blood leukocytes in mice with alloxan diabetes. Biull Eksp Biol Med. 1995,120:33-5.
44. Talukdar S, Oh DY, Bandyopadhyov. Neutrophils mediate insulin resistance in mice fed high fat diet through secreted elastase. Nat. Med. 2012;18:1407-1412.
45. Kaur G, Sarwar AM, Ather M. Nimbidum suppresses functions of macrophages and neutrophils: Relevance to its anti-inflammatory mechanism. Phytotherapy Research. 2006;18(5):419-424.
46. Udeinya IJ, Mbah AU, Chijioke GP, Shu EN. An antimalarial extract from neem leaves is antiretroviral. Trans. Res. Soc. Trop. Med. Hyg. 2004;98(7):435-437.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=768&id=5&aid=7666