Original research paper

Modulation of Immunological and Hematological disturbances of diabetes mellitus by Diets containing combined leaves of *Vernonia amygdalina and Gongronema latifolium*

ABSTRACT

Background: Immunological and hematological alterations often occur in diabetes mellitus as a result of oxidative stress. The study investigated the effects of consumption of diets containing *Vernonia amygdalina* leaves on some makers 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. **Method**: The design consisted of eighty rats randomly divided into eight groups of 10 rats per group. Groups 2, 3, 4, 5, 6,7and 8 were diabetic, while group 1was normal control. Group 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. R**esults:** 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 RBC, hemoglobin, and lymphocyte counts relative to the diabetic control. All the diets resulted in significant (P<0.05) reduction in the level of WBC, platelets, neutrophil, and $CD4^+$ 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 with the combined leaf diet performing better due to positive synergy among the leaves bioactive agents and may be recommended as dietary therapies for management of immunological and hematological complications in diabetes mellitus.

Key words: Diabetes, Diet, *Vernonia amygdalina*, *Gongronema latifolium*, immunological, hematological, cardiovascular risk.

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 diabetes worldwide in 2011 [3]. It is predicted that about 366 million people are likely to be diabetic by the year 2030 [4]. Report has it 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 diabetes and dietary pattern [5]. Many of these studies have reported that vegetable intake reduces the onset of diabetes 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 with regards to the implication of combining several plants or plants components in diet for diabetes 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 Streptozotozin induced experimental diabetic Wistar rats, with the view to evaluating their potential role 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 diabetes mellitus 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.

MATERIALS AND METHODS

Collection and processing of plant materials

Fresh but matured leaves of *Vernonia amygdalina Del* 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 well dried. Dried leaves of *Vernonia amygdalina Del* 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- 8^0C until used for the preparation of rat chow.

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 [12]. (Table 1). Seven (7) different diets were

formulated namely: Control, VA-5%; GL-5%, VA+GL-5%, VA-7.5%, GL-7.55, VA+GL-7.5%. 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.

Animals

Eighty (80) albino rats of Wistar strain (female only) weighing between 83-121g 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, 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 $\pm 5^{o}$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.

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 (12hrs) prior to induction of diabetes. The weight of individual rats were measured and noted. Diabetes mellitus was induced 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) 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 chemotherapeutic agent was introduced for comparison. Insulin dose used was 5U/kg body weight (b.w), given subcutaneously (s.c) according to [14]. It was given once per day post prandial. 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.

Collection of sample for analysis

At the end of the 28 days, food and water were withdrawn. They 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 12h of collection.

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, differential WBC (lymphocytes and mixed), red cell indices MCHC, MCH and MCV), were estimated using the Sysmex® Automated Analyzer KX-2IN, Sysmex Corporation, Kobe-Japan.

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^{0}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.

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.

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 Table 2 and 3 respectively. The results in Table 2 showed that the diabetic untreated rats (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 white blood cells 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* 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 HCT 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 HCT 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 HCT 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 HCT 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* but no pattern was followed by *Gongronema latifolium.*

Table 3 showed that the diabetic control had neutrophil, basophil, monocyte and CD_4^+ cell count that were significantly higher (P<0.05) compared to the normal control (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 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 those 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. This brought their levels to be numerically equal to those of the normal control; and this treatment was also better than insulin particularly at 5% inclusion level.

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 (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 the white blood cells count, neutrophil, lymphocyte, platelet and $CD4^+$ cells of the diabetic untreated rats (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 (RBC) and of recent, even 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 diabetes contributed to the observed increase in WBC count. This is in agreement with [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 red blood cell count, hemoglobin concentration, packed cell volume 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 group and treatment groups. The low hemoglobin concentration in diabetic control group may be associated with kidney damage due to diabetes. 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 diabetes mellitus.The increase in the level of platelet in the diabetic control group may be due to inflammatory responses caused by diabetes 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 diabetes [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 compared to diabetic control. For red blood cells, this gives an indication that the diets could stimulate the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells [28]. This hormone stimulation enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC [29] This may also suggest the restoration of oxygen carrying capacity of the blood. 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 diabetes and thus improve the metabolism and well being of the red cells. The leaves rich content of blood formatting minerals and vitamins [30] might have also help to supplement those lost due to urinary excretion and this might have promoted the formation of RBC and hemoglobin. Red blood cell counts can be a factor in erythropoietin process [31]. The increased circulating erythropoietin concentrations [31] might have been 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 [34] who reported the reversal of anaemia in cadmium toxicity after the supplementation of diet, and [6] who also reported reversal of anaemia in diabetic rats treated with some leafy vegetables. Some parameters such as WBC and neutrophil responded to the leaves in the diet. The mechanism is not known but may relate to the level of the bioactive agents found. Failure of the leaf diet to influence the parameter at low dose level might be due to inadequate amount of the phytochemicals / nutrients needed to reverse the changes in those parameters. Combining the two leaves resulted in better performance indicating a positive synergy. The two leaves contain varying levels of flavonoids, tannins, saponins, polyphenols, alkaloids, hydrocyanic acid, vitamin A, E, C, riboflavin, thiamin, niacin, iron, selenium, and chromium. Combination of the two leaves in diet tended to synergies each other and the result obtained was better with combined leaves than using either of the leaves. Combination of the leaves in a diet at 5% impacted better than insulin on all parameters measured.

Table 3 shows that the neutrophil, basophil, monocyte and CD_4^+ *ce*ll 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 diabetes 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 diabetes 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 diabetes. 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 main effectors cells of the immune system. The increase in the lymphocyte count in rat 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 reduce 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.

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 diabetes with the combined leaf diet performing better due to positive synergy among the leaves bioactive agents. This could be due to the prevention or inhibition of lipid peroxidative system by their antioxidant, 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 diabetes.

COLLEGE ETHICAL COMMITTEE PERMIT REFERENCE NUMBER: UC/PGP/BMEC/11/1/01.

COMPETING INTERESTS

There are no competing interests.

AUTHORS' CONTRIBUTIONS

H. Akpan was involved in conception, design, acquisition of data and drafting the manuscript. **Ekaidem, itemobong** 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.

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Table 1: Percentage composition and nutrient analysis of diets

Feed	**Diets:**

ingredients	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	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	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	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	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	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 ±15.38	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	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)