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Ameliorative Effect of *Gongronema latifolium* Leaf Diets on Hematological and Immunological Disturbances in Streptozotozin-induced Diabetic Rats

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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 GSE 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.

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ABSTRACT

Aim: The aim of this study was to determine some biomarkers of immunology and hematology in streptozotocin- induced diabetic wistar rats consuming *Gongronema latifolium* leaf diets in order to evaluate the involvement of the diet in the management of immunological and hematological complications common among diabetics.
Study Design: The design consisted of fifty rats randomly divided into five groups (1, 2, 3, 4 and 5) of 10 rats per group. Group 1(normal control) was fed with control diet; Group 2 (diabetic control) was fed with control diet; Group 3 and 4 (diabetic, diet treated) were fed with *Gongronema latifolium* at 5% and 7.5% respectively. Group 5 (diabetic, insulin treated) was fed with control diet and treated with insulin. Feed and water were given *ad- libitum* for 28 days.
Place and Duration of Research: The Research took place at the Department of Biochemistry,

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University of Calabar, Nigeria between March, 2011 and May, 2012.
Methodology: Biomarkers of hematology and immunology were determined using standard methods.
Results*:* Results *showed* that diabetic rats in groups 3 and 4 consuming *Gongronema latifolium* diet had significant increase (*P* = .02) in the RBC, hemoglobin, and lymphocyte counts relative to the diabetic control. Diabetic rats consuming *Gongronema latifolium* had significant reduction (*P* = .02) in the level of WBC, platelets, neutrophil, and CD_4^+ cell count relative to the diabetic control. The results for diabetic rats in groups 3 and 4 consuming *Gongronema latifolium* diets were similar to those on Insulin (group 5) for the measured parameters and were not significantly different when compared to the normal control rats (group 2).
Conclusion: We concluded that consumption of diets containing *Gongronema latifolium* leaves has positive effect on the immunological and hematological abnormalities associated with diabetes mellitus and might constitute a diet- based treatment or adjunct for treatment of immunological and hematological disturbances common among diabetics.

Keywords: Diet; diabetes; Gongronema latifolium; immunological; haematological.

ABBREVIATIONS

GL- Gongronema latifolium; HGB- hemoglobin; HGT- hematocrit; PVC- packed cell volume; RBC- red blood cell; WBC- white blood cell.

1. INTRODUCTION

Gongronema 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 [1,2] Guinea Bissau and western Cameroun. As a vegetable, it is used in the preparation of many African dishes. In traditional folk medicine, the leaf is used for treatment of diabetes and hypertension [3,4] as well as for treatment of typhoid fever [3,4]. It is also used to dispel stomach upset and pains [5] and to enhance the return of menstrual cycle [4]. Scientific studies have established its chemical [6] and phytochemical compositions [7,8]. Aqueous and methanolic extract of *Gongronema latifolium* were found to exhibit antibacterial activity against a host of bacteria [6]. Ethanolic extract of *Gongronema latifolium* [9] is antiulcer, analgesic and antipyretic. Aqueous extract of dried leaves of *Gongronema latifolium* were found to exhibit anti-inflammatory activity [10]. Its antioxidant [11,12,13] and antitussive properties in the treatment of fowl coughs [12] have been reported. Reports is also available on the hematological changes following oral administration of ethanolic root extract of *Gongronema latifolium* [7] and the effect on serum protein, hemoglobin, cholesterol, lipid peroxidation, white blood cells, antioxidant enzymes such as glutathione-S-transferase, superoxide dismutase, and liver function enzymes namely alanine transaminase, aspartate transminase and alkaline phosphatase of normal rats on long term consumption of a diet supplemented with leaves of *Gongronema latifolium* [13]. Histological changes of the liver, intestine and testes following chronic dietary intake by normal rats have also been examined [13].

Limited information is available on the antidiabetic activity of *Gongronema latifolium*. Ugochukwu and Babady [2] investigated the effect of aqueous and ethanolic extract of *Gongronema latifolium* leaves on glucose and glycogen metabolism in the liver of normal and streptozotocin-induced diabetic rats. The results showed that the ethanolic extract had antihyperglycemic potency which was suggested to be mediated through the activation of hexose kinase, phosphofructokinase, glucose-6-phosphate dehydrogenase and inhibition of glucose kinase in the liver [2]. The effect of the leaf extract of *Gongronema latifolium* in the management of diabetic lipid peroxidation was reported by Nwanjo [11]. It was found to exhibit anti-lipid peroxidative activity. Edet *et al*. [14] investigated its effect on some cardiac enzymes of alloxan-induced diabetic rats and concluded that *Gongronema latifolium* leaf extract were not hepatotoxic and likely to be of significance in the management of cardiovascular complication in diabetic and non- diabetic users. There is no information on the effect of consumption of diets containing *Gongronema latifolium* leaves in the management of diabetes mellitus. Many reports have appeared in recent years showing that vegetable intake reduces the onset of diabetes

and improves plasma glucose control in diabetic patients. The present study was designed to investigate the effect of consumption of diets containing *Gongronema latifolium* leaves on some immunological and hematological parameters of Streptozotozin induced diabetic rats so as to evaluate its effects in the management of immunological and hematological complications common among diabetics. Immunological and hematological disturbances common in diabetics is a consequence of oxidative stress [15]. Since *Gongronema latifolium* leaves contain valuable antioxidant, especially antioxidant vitamins including ascorbic acid, α- tocopherol, β-carotene and phenolics [16,17], it is suspected that consumption of the leaves might reduce oxidative stress and have some positive effect on immunological and hematological status in diabetes mellitus. This study is justified because it will provide information on the possible dietary role of *Gongronema latifolium* in the management of immunological and hematological disturbances in diabetes mellitus, which may present a household, more available and accessible prophylactic and therapeutic options for diabetics in African countries where the vegetable is popularly used in the preparation of many dishes.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Materials

Fresh but matured leaves of *Gongronema latifolium* were collected from the Endocrine Research Farm, University of 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 processed to powder by the method of Akpan and Ekaidem, [18] and stored in a well – labeled amber container in the refrigerator at temperature 2- 8°C until used for the preparation of rat chow.

2.2 Formulation of Experimental Diets

Standard rat chows (growers) were formulated according to the nutritional requirement of rat [19] (Table 1). Three (3) different diets were formulated namely: Control, 5 % GL (diet containing 5 % *Gongronema latifoli*um leaves) and 7.5 % GL (diet containing 7.5% *Gongronema latifolium* leaves). All diets were isocaloric and isonitrogenous. The percentage composition and nutrient analysis of the experimental diets are shown in Table 1.

2.3 Animals

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. The animals were acclimatized for two weeks using the method of Akpan and Ekaidem, [18]. 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 Canadian Council on Animal Care: Guide to the care and use of experimental animals [20]. Animals were allowed free access to water and chow over a two week adaptation period and closely monitored.

2.4 Experimental Design and Induction of Experimental Diabetes Mellitus

The experimental design and induction of experimental diabetes mellitus were done according to the method of Akpan and Ekaidem, [18]. Fifty (50) female rats were divided into 4 groups of diabetic and 1 groups of normal rats with 10 animals in each group. Prior to grouping, the rats constituting the diabetic groups were subjected to an overnight fast (12hrs) before 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. Ten (10) 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, California, U.S.A) 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 % *Gongronema latifolium* diet, **5 % GL**) was fed with 5% *Gongronema latifolium (GL*) diet

Table 1. Percentage composition and nutrient analysis of diets

Feed ingredients	Diets		
	Control	**GL-5%**	**GL-7.5%**
Soybean meal	33.78	31.03	30.53
Garri	26	25	25
Maize meal	38	37	35
L-Lysine	0.18	0.18	0.18
L-Methionine	0.17	0.17	0.17
Min/vitamin	0.25	0.25	0.25
DCP	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00
Corn oil	0.25	0.25	0.25
G. latifolium	-	5	7.5
Analysis;			
CP	18.40	18.40	18.46
CFAT	4.30	4.14	4.16
CFIBRE	3.71	4.15	4.41
ME	3219	3218	3218

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

Group 4 (diabetic treated with 7.5% *Gongronema latifolium* diet, **7.5%GL**) was fed with 7.5% *Gongronema latifolium (GL*) diet

Group 5 (diabetic treated with Insulin, **INSD**) 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 Sonia and Srinivasan [21]. It was given once per day post prandial. Treatment lasted for 28 days.

2.5 Collection of Sample for Analysis

At the end of the 28 days, food and water were withdrawn and the rats were allowed to fast overnight. The following day, the rats were 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.

2.5.1 Determination of Full Blood Count (FBC) using automated hematology analyzer, KX-2IN (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.

2.5.2 CD_4^+ count

The CD_4^+ lymphocyte was estimated by flow cytometry [22] 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.

2.5.3 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 diets containing *Gongronema latifolium* on some biomarkers of

hematology and immunology of diabetic rats are shown in Tables 2, and 3 respectively. Table 2 shows that the diabetic control had significantly (*P* = .02) 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). RBC was significantly (*P* = .01) lower for the diabetic control rats (6.13 ± 0.14 x10^6/UL) compared to the normal control (7.01 ± 0.13 x10^6/UL). HGB was also significantly (*P* = .01) lower for the diabetic control rats (12.30 ± 0.27 gm/ml) compared to the normal control (13.40 ± 0.04 gm/dl). HCT was also significantly (*P* = .001) lower for the diabetic control rats (30.13 ± 0.73%) compared to the normal control (45.13 ± 0.34%). The PLT was significantly higher (*P* = .02) for the diabetic control rats (715 ± 39.61x 10^3/µl) compared to the normal control (619 ± 19.08 x 10^3/µl).

When the diabetic control was compared with groups receiving treatments, it was observed that rats consuming 7.5 % *Gongronema latifolium* diets had significant (*P* = .02) reduction in WBC count (8.33 ± 0.44 x 10^3/µl) relative to diabetic control (10.60 ± 2.00 x 10^3/µl). Diabetic rats treated with insulin showed no significant (*P* = .06) reduction in WBC count (7.43 ± 0.33 x 10^3/µl relative to diabetic control (10.60 ±2.00 x 10^3/µl). The diabetic rats consuming *Gongronema latifolium* diets had significant (*P* =.02) increase in RBC, significant (*P* = .001) increase in HGB, and significant (*P* = .001) increase in HCT relative to the diabetic control. Those on 5per cent GL had RBC, HGB, and HCT of 7.00±0.22 x10^6/UL, 18.23±0.26 gm/dl, and 45.13±1.44% respectively, while those on 7.5 percent GL had RBC, HGB and HCT of 7.64±0.18 x10^6/UL, 18.40±0.43 gm/dl and 46.83±1.38% respectively. Diabetic rats treated with insulin had RBC, HGB and HCT of 6.48±0.33 x10^6/UL, 12.43±0.58 gm/dl, and 41.73±1.45% respectively. For the diabetic rats on insulin, HGB count was significantly higher (*P* = .001), HCT count was also significantly higher (*P* = .02) and RBC count was also significantly higher (*P* = .001) when compared to the diabetic control. The PLTs of the diabetic rats consuming 5% *Gongronema latifolium* was significantly lower (*P* = .04) compared to the diabetic control. 5% GL had 6.77±58.18 x10^3/µl, 7.5% GL had 6.28±60.36 x 10^3/µl. Those on insulin had 6.04±18.21 x 10^3/µl. The RBC functional indices MCV, MCH, and MCHC followed similar pattern as RBC (Table1).

Table 3 shows that there was a significant (*P* = .001) increase in basophil, significant (*P* = .02) increase in monocyte and significant (*P* = .001) increase in CD_4^+ cell count for the diabetic control (1.81±0.21%, 5.40±0.00 %, and 51.53±0.77 cell/µl respectively) compared to the normal control (0.00±0.00%, 4.67±0.33 %, and 15.70±0.36 cell/µl respectively). The neutrophil count increased in the diabetic control group but the increase was not significant (*P* = .09) compared to the normal control. The eosinophil was significantly (*P* = *.04*) lower and the lymphocyte was also significantly (*P* = *.001*) lower for the diabetic control (1.66±0.55%, and 43.33±4.21% respectively) compared to the normal control (2.67±0.56%, and 70.67±2.19% respectively).

Treatment with the diets significantly (*P* = .02) lowered the neutrophil, monocyte and the CD_4^+ cell count relative to the diabetic control. Treatment with the diets significantly (*P* = .01) increased the lymphocyte compared to the control. The same results were obtained for insulin. But, only treatment with insulin significantly (*P* = .05) increase the eosinophil; treatment with diet non- significantly (*P* =.42) increase the eosinophil relative to the diabetic control.

Plant based food diets have advantages in the prevention and treatment of chronic diseases, including diabetes mellitus. At present, there are few studies on the effect of vegetarian diet in diabetes. Akpan and Ekaidem [18] has recently reported the modulatory role of diet containing combined leaves of Vernonia amygdalina and Congronema latifolium on hematological and immunological disturbances in diabetic rats. In this study, we assessed the effect of consumption of diets containing *Gongronema latifolium* leaves on hematological and immunological parameters of streptozotozin induced experimental diabetic Wistar rats, with the view to evaluating its involvement in the management of hematological and immunological complications common among diabetics.

Table 2 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 be a manifestation of the diabetic condition. This is in line with normal physiologic response following the perception of an assault by the body. It is likely that the damage caused by diabetes contributed to the observed increase in WBC count. This is in agreement with Finlayson et al. [23].

Table 2. Effect of dietary consumption of *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%GLD	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]
7.5%GLD	8.33±0.44[a]	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]
INSD	7.43±0.33[a,b]	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 dietary consumption of *Gongronema latifolium* on differential white blood cell count and $CD4^+$ cells 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,c]	1.33±0.55[b,d]	1.81±0.00	43.33±4.21[b]	5.40±0.00[b]	51.53±0.77[b]
5%GLD	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]
7.5%GLD	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]
INSD	26.00±5.17[a]	5.00±0.73[c]	0.00±0.00	59.00±0.22[a]	4.00±0.00[a]	17.87±0.51[a]

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

who reported that leucocytosis may occur in hepatic damage. White blood cell count is increased in obesity [24] and is a risk factor for atherosclerosis [25]. An elevated WBC count is present in impaired glucose tolerance (IGF) [25] and is associated with macro- and micro angiopathic complications in type 2 diabetes [26]. High WBC predicts the development of type 2 diabetes and is associated with decrease in insulin action [27].

The red blood cell count, hemoglobin concentration, packed cell volume alongside with erythrocyte function indices: MCV, MCH and MCHC all reduced significantly in the diabetic control compared to the normal group and treatment groups. The reduction in red blood cell count may be due to an increase in the production of lipid peroxides that lead to hemolysis of RBC [28]. The major pathological consequences of free radical induced membrane lipid peroxidation include increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity [29]. It may also be due to reduction in the synthesis of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells. Erythropoietin is produced by the kidney. Kidney damage at several levels is a complication of diabetes. Changes in the kidney that occur with diabetes range from diabetic nephropathy all the way to chronic kidney disease (CKD).

The low hemoglobin concentration in diabetic control group may be associated with kidney damage due to diabetes. Low hemoglobin concentration was associated with low postprandial c-peptide concentration and low β-cell responsiveness. Some studies suggest that β-cell dysfunction occurs due to hypoxic damage to the pancreatic islet cells [30,31], which progressed to diabetes and low hemoglobin concentrations contributing to development of cardiovascular events in patients with diabetes [32]. Diabetes mellitus is associated with a more rapid decline in glomerular filtration rate than that of other kidney diseases [32] and diabetic nephropathy and diabetic retinopathy result in increased susceptibility to low hemoglobin level [33]. Low hemoglobin level is associated with increased cardiovascular mortality and chronic kidney disease (CKD) in patients with diabetes mellitus [34].

Hematocrit (PCV or HCT) is an indication of the percentage of red blood cells in the blood. Reduction in the PCV points to reduction in kidney function that occurred due to diabetes which can result in lower hematocrit. The decrease in MCV, MCH and MCHC values, observed in the diabetic control group may be an indication of abnormal hemoglobin synthesis, failure of blood osmoregulation, and plasma osmolarity [35].

The level of platelet in this study was significantly higher in the diabetic control compared to the normal group and treatment groups. Increase in the level of platelet is due to diabetes and correlate with the level and sustenance of high blood glucose level. Platelets assume an important role in signaling of the development of advanced atherosclerosis in diabetes [36,37,32]. Platelets are small discoid blood cells that circulate and participate in hemostasis. Primary plug formation due to platelets seals the vascular defects and provides the required phospholipid surface for the recruited and activated coagulation factors [36]. In response to stimuli generated by the endothelium of blood vessels, platelets change shape, adhere to subendothelial surfaces, secrete the contents of intracellular organelles, and aggregate to form a thrombus [36]. These pro-aggregatory stimuli include thrombin, collagen, epinephrine, ADP (dense storage granules), and thromboxane A2 (activated platelets) [36].

We observed that consumption of diets containing *Gongronema latifolium* leaves significantly reduced WBC and platelet but significantly increased RBC, HGB, PCV, MCV, MCH and MCHC compared to diabetic control. This gives an indication that the diets at the two inclusion levels can stimulate the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells [38]. This hormone stimulation enhances rapid synthesis of RBC which is supported by the improved level of MCH and MCHC [39]. This may also suggest the restoration of oxygen carrying capacity of the blood, hence the increased hemoglobin concentration and packed cell volume. Although the mechanism of this effect is not well known, it is believed that the antioxidants in the leaf [8] might have help to reduce oxidative stress by mopping up free radicals caused by diabetes. The leaf rich content of iron and vitamins [8] 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 [40]. Increase in red blood cell count

following consumption of *Gongronema latifolium* diets might have resulted in increased rate of erythropoietin production against the diabetic control. The increased in the concentration of circulating erythropoietin [40] might have help to elicit and enhance the production and expression of red cells antioxidant [41] and their ability to lower lipid peroxidation level [42] and decrease the rate of hemolysis of erythrocytes.

Antioxidants prevent the expression of hepcidin, a 25 amino acid peptide hormone, in the liver which is increased dramatically by inflammation and because of chronic disease [43]. Once released, hepcidin is bound to iron efflux protein ferroportin [44] and act as a negative regulator of body iron homeostasis, inhibiting the release of iron recycled from senescent red blood cells by reticuloendothelial macrophages [45] and the absorption of dietary iron by intestinal enterocytes [46]. Thus, the diets may participate directly to the amelioration of RBC indices and correction of the anemic status in the diabetic rats by attenuating proinflammatory cytokines production [47] which is central to the expression of hepcidin. The result we obtain is in line with the findings of [48] who reported the reversal of anaemia in cadmium toxicity after the supplementation of diet and [15 who also reported reversal of anaemia in diabetic rats treated with some leafy vegetables.

Table 3 shows that the neutrophil, basophil, monocyte and CD_4^+ cell count were significantly increased in the diabetic control group compared to the treatment group and the normal control, while the lymphocyte was significantly reduced in the diabetic control group compared to the treatment group and the normal control. High WBC count has been shown to be associated with insulin resistance, decrease in insulin action, and is predictive of the development of type 2 diabetes [24], coronary artery disease [49], stroke [49], and diabetes micro- and macrovascular complications [26]. 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 [50], oxidative stress [51], angiotensin II [52], and cytokines [53]. The release of cytokines, such as TNF-α [54], tranforming growth factor-1 [55], superoxide [56], nuclear factor κB (NF-κB) [57], monocyte chemoattractant protein 1, interleukin-1β, and others [54] 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 [58]. The raised leukocyte count reflected low-grade inflammation [58]. 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 of WBC mediated by neutrophil to insulin resistance [59]. We suggest that the raised leukocyte count may be a response to the disease- diabetes which might be seen as infection and the mechanism might be connected to leptin and the leptin receptor that are parts of a pathway that stimulates hemopoiesis [60] as well as class I cytokine receptors [61] which includes the gp130 subunit of the IL-6 receptor family [62]. The decrease in the WBC count, in diabetic treated rats shows the anti-inflammatory property of the *Congronema latifolium* leaf diets. The increase in the lymphocyte count in rat placed on *Gongronema latifolium* 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 [63,64]. The effect of the extract was attributed to inhibition of immune cells migration and phagocytosis, particularly for macrophage and neutrophils in respect to inflammatory stimuli. The extract inhibited the induction of inducible nitric oxide synthase, prostaglandins E_2 (PGE_2) and interleukin 1 (IL-I) productions [65], thus controlling the increased vascular permeability associated with inflammatory reactions.

4. CONCLUSION

The findings of the present study indicate that consumption of *Gongronema latifolium* leaf diets ameliorates hematological and immunological disturbances associated with diabetes. This could be due to the prevention or inhibition of lipid peroxidative system by their antioxidants, maintenance of cellular integrity, and attenuation of pro-inflammatory cytokine production. Consumption of diet containing *Congronema latifolium* at the level in this study may be recommended as a dietary therapy or adjunct to main therapy for diabetic immunological and hematological complications.

DISCLAIMER

College Ethical Committee permit reference number : Uc/pgp/bmec/11/1/01.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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