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The Role of Ethanolic Leaf Extract of *Ageratum Conyzoides* in Amelioration of Haematological Parameters in Streptozotocin-Nicotinamide Induced Type-ii Diabetic Rats

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Abstract

Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein, and lipid metabolism and by complications like retinopathy, microangiopathy, and nephropathy. Several haematological changes are shown to be directly associated with DM. This study was designed to investigate the haematological indices of ethanolic leaves extract of *Ageratum conyzoides* in streptozotocin induced diabetic wistar rats. Healthy adult male *Wistar* rats were used for the study and diabetes was induced by intraperitoneal injection of 60 mg/kg streptozotocin and divided into Group I: Negative Diabetic control rats received sterile water orally (as vehicle) daily. Group II: Positive Diabetic (DM) control rats received a standard drug, Glibenclamide (0.25 mg/kg) orally. Group III: Diabetic rats (treated group) received 500mg/kg body weight of crude extract of *Ageratum conyzoides* (DM + AC) orally. Daily water and feed intake were recorded. After day 28 treatments, blood was collected for haematological analysis. There was significant difference in the Packed Cell Volume, Red Blood Cell count, White Blood Cell count and Haemoglobin concentration comparing the different groups. Our data showed that, *Ageratum conyziodes* extract increase feed intake, body weight, red blood cells and packed cell volume of diabetic rats similar to what was observed in glibenclamide treated groups, but more effect was observed in the *Ageratum conyzoides* treated group. We conclude that *A. conyzoides* at the dosage of 500mg/kg like glibenclamide, can proffer better results in ameliorating the haematological indices in diabetic rats at cheaper cost.

Key words: Diabetes mellitus, haematological changes, *Ageratum conyzoides,* glibenclamide

INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder of the endocrine system and characterized by increased blood glucose level resulting from the defects in insulin secretion, action, or both (Irudayaraj *et al.,* 2002). Diabetes mellitus is a worldwide health problem and leads to microvascular and macrovascular complications (Umar *et al.,* 2010). Worldwide, it is expected that about 366 million people are likely to be diabetic by the year 2030 and Nigeria, more than 3.9 million (Lin *et al.,* 2004). Diabetes mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein, and lipid metabolism and by complications like retinopathy, microangiopathy, and nephropathy. Several haematological changes affecting the red blood cells (RBCs), white blood cells (WBCs), and the coagulation factors are shown to be directly associated with DM (Mbata *et al.,* 2015). Haematological abnormalities reported in the DM patients include RBCs, WBCs, and platelet dysfunction, anaemia, decrease oxygen carrying capacity of RBCs and hypercoagulation (Mirza *et al.,* 2012; Bharathi, 2016).. Interest is currently on amelioration of these effects. *Ageratum conyzoides (AC)* is an herb which grows abundantly throughout Nigeria, and known in folkloric medical practice for treatment of various disease conditions (Palmer *et al.,* 2019). It is a common annual herbaceous weed with long history of traditional medicinal use. *Ageratum conyzoides* preparation is a local herbal remedy can be readily available and cheap for human population. Its efficacy as an antibiotic, anti-inflammatory and analgesic agent have been verified (Palmer *et al.,* 2019). Research in Nigeria showed haematopoietic properties of ethanolic leaf extract of *Ageratum conyzoides* in albino rats. Specifically, a dose dependent increased effects was reported on pack cell volume (PCV), haemoglobin (Hb), RBC count, mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). In Cameroon and Congo, it is used to treat fever, rheumatism, headache and colic (Bioka *et al.,* 1993); in Kenya it is also employed as an antispasmodic (Achola *et al.,* 1994). The use of this species in traditional medicine is extensive in Brazil as the aqueous extract of leaves or whole plant is utilized to treat colic, cold and fevers, rheumatism, and it is recommended by Brazilian Drugs Central as an antirheumatic (Galati *et al.,* 2001). However, the effect of *A. conyzoides* on haematological indices in streptozotocin-induced diabetic rats have not yet been elucidated. This study therefore aimed to evaluate the ameliorative effects of A. conyzoides on feed consumption rate, body weight gain and haematological parameters in streptozotocin-induced diabetic Wistar rats.

Materials and methods

Plant Collection and Extraction

The test plant, *Ageratum conyzoides* was collected from the University of Abuja environment and sent to the Botanical unit of the Faculty of Science of the University for proper identification. The plant was washed under running tap water, the leaves were then plucked, and allowed to dry at the temperature of 25^0C in a hood without exposure to direct sunlight. The dried leaves were then grounded into fine powder. Cold extraction method was carried following the protocol described by Ejeh *et al.,* (2019). Briefly, the leave powder was soaked with absolute ethanol for 72 h at room temperature with intermittent rigorous shaking using automated shaker. The extract was concentrated in a hot-air oven at 40^0C and stored at 4^oC until time of use.

Experimental Animals

Healthy adult male *Wistar* rats between 2 and 3 months of age and weighing 200-250g were obtained from the Faculty of Veterinary Medicine experimental animal unit and used for this study. The animals were kept according to experimental groups (six (6) members per group) in metal cages in a well-ventilated animal house of the Veterinary Medicine faculty, University of Abuja, Nigeria with a regular controlled light cycle (12 h light/12 h dark). Food (standard commercial pelletized rat feed) with clean tap water was provided *ad libitum.* Our animal experimental protocol received approval from the University of Abuja Ethics Committee on Animal experiments (UAECAU/2019/018) and was conducted according to ethical standard of ARRIVE guidelines, in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and EU Directive 2010/63/EU for animal experiments.

Induction of non-insulin dependent diabetes mellitus (NIDDM)

Non-insulin dependent diabetes mellitus (NIDDM) was induced following the protocol described by Pellegrino *et al.,* (1998). Briefly, a single intraperitoneal (i.p) injection of 60 mg/kg streptozotocin was administered to each rat and fifteen (15) minutes later, a single i.p injection of nicotinamide at 120 mg/kg was also administered to same rats. Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide dissolved in normal saline. Hyperglycemia was monitored and confirmed by elevation of glucose levels in plasma at 48h, 72h and then 7days post induction. Only rats that are found to have permanent NIDDM were used for the study as diabetic rats.

Experimental design for anti-diabetic study

All diabetic and non-diabetic rats (rats not induced with streptozotocin and nicotinamide) were divided into the following groups of six (6) members per group: Group I: Negative diabetic control rats received sterile water orally (as vehicle) daily for 28 days. Group II: Positive diabetic control rats received a standard drug, Glibenclamide (0.25 mg/kg) orally for 28 days. Group III: diabetic rats (treated group) received 500mg/kg body weight of crude extract of AC orally for 28 days. This dose has been reported to be therapeutic with no toxicological effects (Palmer *et al.,* 2019). Group IV: Non diabetic control rats received sterile water orally (as vehicle) daily for 28 days.

Evaluation of fasting glucose level

Fasting blood glucose levels of each rat in each group was confirmed as described by Pellegrino *et al.,* (1998). Briefly, the tip of the tail of the rat was cut with a sharp scissors and a drop of blood was dropped on the glucose test strip already place in the AccuCheck® glucometer. The readings were taken after the beep sound from the glucometer.

Evaluation of daily body weight

During the experimental period, the rats were weighed daily using a bench-top digital scale (Kerro Ecostar USA, BL-PID/20001 with sensitivity of 0.1g) and the mean change in body weight calculated.

Blood sample collection

Twenty-four hours after the last treatment, blood was drawn from the retro-orbital venous plexus of each animal into EDTA tubes for haematology assay before they were sacrificed by quick cervical method as described by Usende *et al.* (2018a).

Haematology assays

Blood sample collected into EDTA sample tubes was used for the determination of haematological parameters, including red blood cell count, total white blood cell count, and other blood cells and specifically, red blood cell index using standard veterinary automated haemoanalyser (Labomed® ABX Micros ESV 60, USA).

Techniques of Data Analysis

All data obtain from this study was expressed as mean and SEM (Standard Error of the Mean). The significant difference in means between treatment and control groups was determined using one-way analysis of Variance (ANOVA) at P value of 0.05 (Duncan multiple range test as posthoc) using Graph pad prism version 9.

Results

Feed consumption

Feed intake of diabetic and non- diabetic rats was not significantly different ($p>0.05$); Although not significant, there was slight decrease feed intake in DM + NT group compare to diabetic treated group with either Glibencamide or *Ageratum conyzoides* group. However, a constant increase in the feed intake was observed across groups from week 0 to week 4 (Fig. 1).

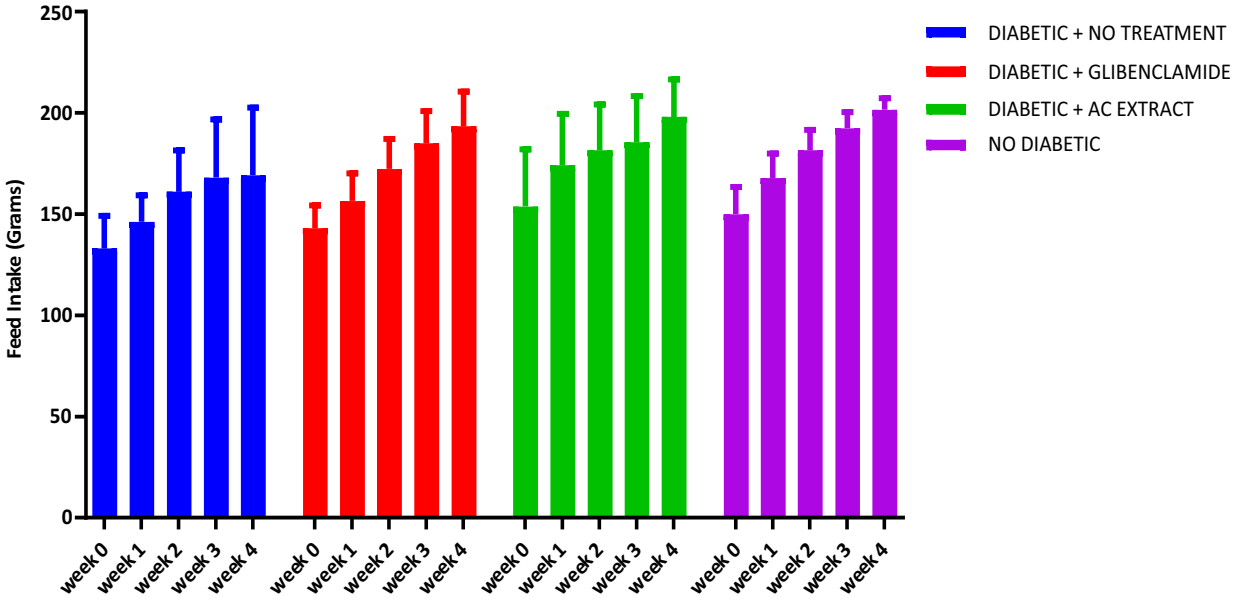

Fig 1: Bar chart showing the weekly feed intake of diabetic groups and non-diabetic group with constant increase in feed intake from induction of DM to week 4 of treatment. No statistically significant difference was noticed.

Body Weight

The body weight of wistar rats in each group were measured weekly and subjected to statistical analysis. There was no significant difference in the weekly body weight of diabetic groups and normoglycaemic rats (Fig. 2). However, there was gradual gap between the DM + NT comparing to other groups (NO DM, DM + GLI and DM + AC) from week 1 all through to week 4 of the study with the DM +NT group having the least weight gain. At week 3 and week 4, there was no difference in body weight of the DM + NT which indicates no weight gain for the week. For the DM + AC treated rats, there was a constant weight gain from week 2 to week 3 with no mean weight loss. Interestingly, The DM +AC group has higher weight compared to the DM +GLI and the NO DM groups (Fig. 2).

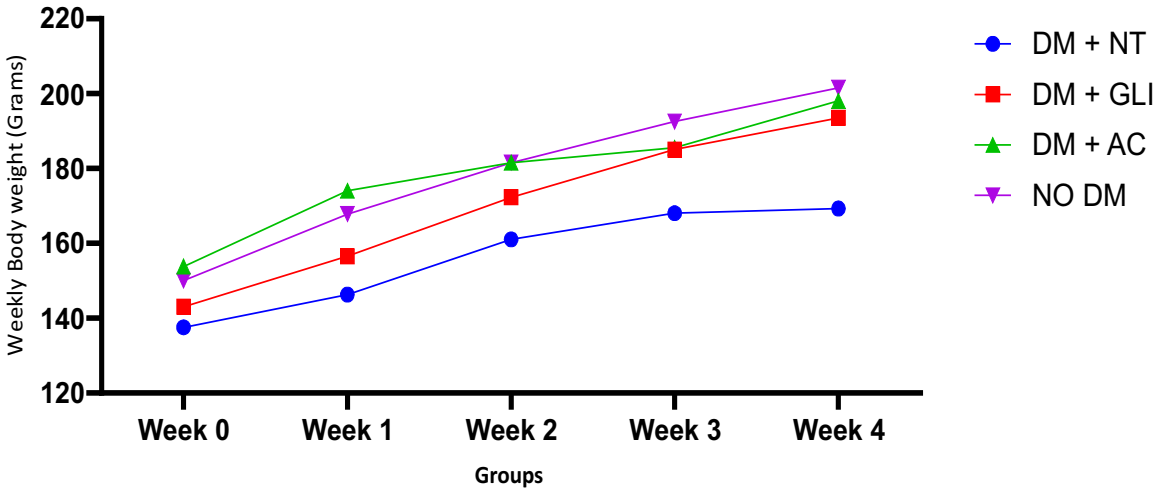

Fig 2: Line graph showing the weekly body weight in gram of the diabetic groups and normoglycaemic group, although not statistically significant but prominent is the DM + NT group with less steeply graph compared with the other groups (NO DM, DM + GLI and DM + AC).

Weekly Glucose Result

Figure 3 showed the weekly glucose level of the diabetic groups and normoglycaemic group, at weeks under study. The Diabetic group has blood glucose level above the 200mg/dl reference range for diabetic patient and the No DM group has a blood glucose level below the 200mg/dl. At week 1, 2, and 3, the Diabetic groups still has blood glucose level above 200mg/dl. At the end of the study at week 4, the DM+GLI and DM+AC had reduced blood glucose level below the 200mg/dl mark indicating an effect of glibenclamide as a standard drug for treatment of Diabetes Mellitus and also AC extract as a potential treatment for DM.

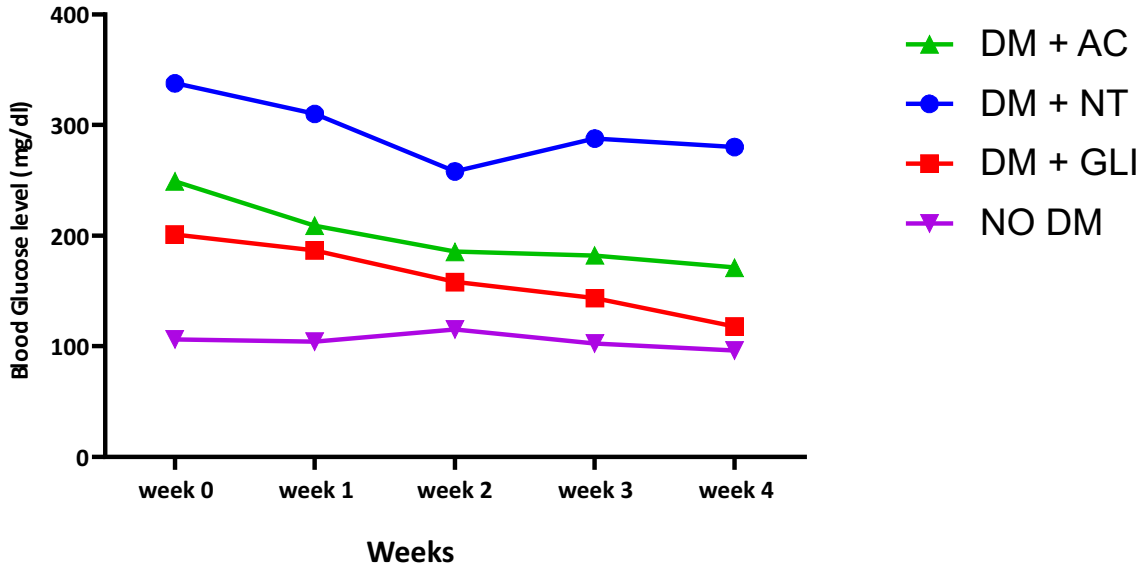

Fig 3: Line graph showing the weekly glucose level of during the period of experimentation

Haematological Results

The haematological parameters were analysed and expressed in mean±SEM per group and multiple comparison inputted as a superscript on the values and shown in table 1. The White blood cell counts was significant when comparing No DM group with DM + NT group ($p>0.05$) with increased white blood cell counts in the Diabetic without treatment group. Although not significant, there is ameliorative effect on the WBC counts in the standard treated groups with glibenclamide for 28 days. The AC extract treated group shows similar trends with the glibenclamide treated group with WBC value lower than the standard drug group.

Also, there was significant difference observed when comparing the RBCs parameter of No DM group with DM + NT group ($p>0.01$), DM + GLI group ($p>0.01$) and DM + AC group ($p>0.01$). Increased RBC count was observed in the Diabetic groups compared to the non-diabetic rat groups especially the DM + NT group. The RBC count of DM + AC was also slightly higher than the RBCs of group treated with standard drug but not significant.

The haemoglobin concentration of No DM with positive diabetic control significantly differs ($p>0.05$) with increase observed in the positive diabetic control group. Glibenclamide or AC extract slightly corrected the haemoglobin concentration observed in the DM + NT group but not significant.

Mean Corpuscular Volume (MCV) is a measure of size of Red Blood Cells during determination of haematological parameter, there was significant difference when No DM was compared with DM + NT ($p>0.05$), DM + GLI ($p>0.05$) and DM + AC ($p>0.05$). The significant difference was due to increased MCV in the No DM group.

Although not significant, there was increased Packed Cell Volume (PCV) in the DM + NT group compared to the other groups. Platelet was also observed to be high in DM +AC group but not significantly relevance.

Parameters	Groups			
	DM + NT	DM+ GLI	DM + AC	No DM
White Blood Cell	26.20±3.26	18.20±0.52	17.35±3.74	12.93±1.55[a]
Red Blood Cell	10.07±0.22	9.75±0.29	9.83±0.27	8.45±0.18[a, b, c]
Haemoglobin	16.98±0.29	16.33±0.57	16.43±0.53	15.13±0.49[a]
PCV	52.75±1.11	50.25±1.60	51.00±1.47	47.50±1.66
MCV	52.48±0.86	51.60±0.61	52.05±0.67	56.28±0.65 [a, b, c]
MCHC	32.10±0.35	32.40±0.18	32.13±0.24	31.75±0.08
Platelets	491.00±99.81	494.5±35.39	580.30±34.77	465.00±16.35
Neutrophil	2.25±0.48	1.25±0.25	4.50±1.85	3.75±0.85
Lymphocytes	85.25±2.49	85.00±0.91	80.00±1.58	63.25±19.11
Eosinophils	0	1.25±0.48	0.25±0.25	0.5±0.29
Basophils	0.25±0.25	0	0	0

Table 1: Table showing the haematological parameters of the diabetic groups (DM + NT, DM + GLI and DM + AC) and no diabetic group (No DM). The result was subjected to statistical analysis with multiple comparison across groups using Tukey as posthoc test. [a] - when No DM significantly differs with DM + NT, [b] – when No DM differs significantly with DM + GLI, [c] – when No DM differs significantly with DM + AC group.

Discussion

It is now well recognized that the abnormal metabolic state that accompanies diabetes is responsible for vascular dysfunction, and these abnormalities may include chronic hyperglycaemia, dyslipidaemia, and insulin resistance (Ferroni *et al.,* 2004). All these factors render arteries susceptible to atherosclerosis, being capable of altering the functional properties of multiple cell types, including endothelium and platelets (Colwell *et al.,* 1983).

In diabetes mellitus, feed intake has been shown to increase in diabetic rats than normoglycaemic rats (Oyedemi *et al.,* 2011) which is a symptom in type 2 diabetes in animal and human model. Interestingly, we observed herein a similar result reported by Oyedemi *et al.,* (2011), with increase in feed intake in diabetic group compare to the normoglycaemic rats which was however not significant. The increased feed intake might be due to availability of excess glucose in the blood

without efficient insulin to store into glycogen, this tricks the diabetic rats into consuming more food. Interestingly *Ageratum conyzoides* or glibenclamide treated groups shows the same trend with the increase feed intake making this plant a good candidate in diabetes treatment.
In checking feed conversion rate, the body weight is very important parameter to consider. In DM + NT, there is slight increase in body weight of the affected individual which has also been observed by Oyedemi *et al.,* (2011) and Cintra *et al,* (2017). Interestingly, there was obvious body weight increase in the *Ageratum conyzoides* or glibenclamide treated groups with better result observed in the *Ageratum conyzoides* treated group, probably due to increase intake.

In diabetes mellitus, the haematological indices are very important as well as the blood glucose level. *Ageratum conyzoides* has been reported by Ita *et al.,* (2007) to have haematopoietic effect on the bone marrow, and our findings reported herein showed a positive correlative effect with significant increase in RBC observed in diabetic rats treated with *Ageratum conyzoides* extract compared to non-diabetic group and diabetic treated with glibenclamide as standard drug. The significant increase in RBC observed might be as a result of the haemopoetic properties of *Ageratum conyzoides* which was also reported by Ita *et al.,* (2007). However, we observed significant increase in RBCs of DM + NT which is unusual and remains to be investigated. We however hypothesized that this could be due to the medication used to indice DM.

A significant increase in WBCs was observed in DM + NT group compared to the No DM group, which is a features of diabetes mellitus and also reported by Colak *et al.,* (2014). In the diabetes group treated with either *Ageratum conyzoides* or glibenclamide, WBCs were reduced close to the No DM group which reflect response to treatment, although, no significant difference occurred between the glibenclamide and the *Ageratum conyzoides* extract treated group. The increase in total leucocytes count in diabetic group might be due to increased lymphocytes count in diabetic rats (Colak *et al.,* 2014). We also reported herein that haemoglobin concentration significantly increases in the positive diabetic control group compared to No DM group similar to earlier report of Al-Ali (2016), and that the packed cell volume (PCV) of *Ageratum conyzoides* treated group have increased PCV but not significant. Similar findings concerning PCV have also been reported by Al-Ali, (2016) with no significant difference in diabetic and non-diabetic groups. However, WBC count and haemoglobin concentration were found to be significantly increased ($p< 0.01$) in diabetic rats than non-diabetic rats (Al-Ali, 2016) in a cross-sectional study in diabetes patients with long period of suffering from diabetes. We report herein similar findings.

In conclusion, *Ageratum conyziodes* extract just like glibenclamide increase feed intake, body weight, red blood cells and packed cell volume of diabetic rats. Interestingly, these effects were better observed in the *Ageratum conyzoides* extract group. Also, the ameliorative properties of *Ageratum conyzoides* was observed more in correcting the increased haemoglobin concentration and the white blood cell in diabetic rats than the glibenclamide treated group. Thus *A. conyzoides* at the dosage of 500mg/kg can proffer better results comparative to glibenclamide in ameliorating the haematological indices in diabetic rats and at a cheaper cost.

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Competing Interests
The authors have no financial or non-financial interests to disclose.

Author Contributions
All authors contributed to study design and conception. Material preparation, data collection and analysis were performed by Usende Ifukibot Levi, Ejeh Sunday Augustine and Mobolaji Abdulateef Ayoola. The first draft of the manuscript was written by Mobolaji Abdulateef and Usende Ifukibot Levi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Funding was acquired by Mobolaji Abdulateef

Ethics approval
Animal experiments received ethical acceptance from University of Abuja Ethics Committee for Animal Use (UAECAU/2019/018) and according to ethical standard of ARRIVE guidelines, in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and EU Directive 2010/63/EU for animal experiments.

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