

*Full Length Research Paper*

## **Effect of ethanolic leaf extract of *Vinca major* L. on biochemical parameters and glucose level of alloxan induced diabetic rats**

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In view of World Health Organization (WHO) recommendation on the use of medicinal plants for the management of diabetes mellitus, this study evaluated the effect of ethanolic leaf extract of *Vinca major* L. on biochemical parameters and glucose level of alloxan induced diabetic rats as objective. Sixty-four male Wistar albino rats were induced with diabetes by a single intraperitoneal injection and were separated into four groups (1-4) of sixteen rats each. Group 1 served as the control while groups 2 to 4 served as the test groups. Apart from the control, the test groups were treated with different concentrations of the leaf extract, and four rats from each group were sacrificed every seven days for assessment. The treatment period lasted for 28 days. Haemoglobin (Hb) levels in groups 3 and 4 rats significantly increased ( $p < 0.05$ ) against the control throughout the number of days of the study. Red blood cell (RBC) levels in groups 2 and 4 increased significantly ( $p < 0.05$ ) against the control on the 14<sup>th</sup> day, while all the test group rats had significantly increased ( $p < 0.05$ ) RBC levels on the 21<sup>st</sup> and 28<sup>th</sup> days of the study. The observed trend followed by electrolyte ions, urea and creatinine in test rats against the control in the present study, may be attributed to *V. major* leaf extract trying to salvage the excretory organs of alloxan battered diabetic rats. Glucose level, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) liver enzymes reduced significantly ( $p < 0.05$ ) in test rats against the control. Diabetic rats treated with *V. major* leaf extract in this study, showed significant improvements on those associate problems of diabetes which include anaemia, dyslipidaemia, and hepatic necrosis and inflammation. Rats treated with the leaf extract also showed reduced glucose level (Hypoglycemia). From the observations of this study, extract from leaf of *V. major* may be effective against diabetes and some of its associate problems.

**Key words:** diabetes, biochemical parameters, *Vinca major*, leaf extract, Wistar rats.

## INTRODUCTION

Diabetes is among the diseases that plagued the existence of man on this planet Earth. According to Demoz et al., (2015), diabetes is a complex and chronic illness characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Edem (2009) and Ogbonnia et al., (2008) noted that diabetes is a major degenerative disease in the world today, affecting millions of people and having complications which include hypertension, atherosclerosis and microcirculatory disorders. Demoz et al., (2015) reported that over 3 million deaths worldwide are attributed to diabetes every year. Over 380 million people were reportedly living with diabetes in 2014, and the number is projected to rise by more than double by 2035 without any intervention (Shrivastava et al., 2013). Type 2 diabetes mellitus (T2DM) is among the existing types of diabetic disorder with rapidly increasing incidence in the world (Demoz et al., 2015; Islam et al., 2017). According to Kesari et al., (2007), Type 2 diabetes mellitus accounts for about 90% of diabetic population. Islam et al., (2017) and Bastaki (2005) noted that among all the endocrine disorders, diabetes mellitus is the most prevalent one and almost three hundred million will suffer from the disease by 2025. Ethnopharmacological studies revealed that more than 1200 plants are utilized in traditional medicine for their alleged hypoglycemic activity (Marles and Farnsworth, 1995; Nole et al., 2016; Tsabang et al., 2016). Such plants are known as medicinal plants and are the main source of organic compounds such as polyphenols, tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and etcetera (Marles and Farnsworth, 1995). These compounds are collectively addressed as phytochemicals (Marles and Farnsworth, 1995; Amadi et al., 2012; Duru et al., 2018) and represent a source for the discovery and development of new types of antidiabetic molecules (Firdous, 2014). Nawrot et al. (2003) and Agomuo et al., (2017) noted that natural compounds found in plants or their synthetic forms are the basis of modern pharmacopeia.

*Vinca major* L. an Apocynaceae also known as periwinkle, is one of such medicinal plants with some of these organic compounds addressed as phytochemicals. It is an evergreen perennial trailing vine plant that roots along its stems to form dense masses of groundcover. It can grow up to 25 cm (10 in) high while spreading indefinitely (Blamey and Grey-Wilson, 1989). The leaves of *V. major* can grow up to 9 cm long and 6 cm broad. Its flowers are hermaphrodite in nature (Blamey and Grey-Wilson, 1989). The plant is found along river banks and grows well in full sun and shade at an altitude between 0 and 800 metres (0–2,625 ft) above sea level (Blamey and

Grey-Wilson, 1989). Extract of the plant is used against diseases such as malaria, leukemia and Hodgkin's disease. The leaf juice of *V. major* is used in the treatment of treat wasp stings, and sore throats. The flower extract is used for infants' eyewash. The plant is used in the preparation of periwinkle tea, which is used in the treatment of cough (Blamey and Grey-Wilson, 1989). Due to adverse effects and other factors militating against the use of many synthetic anti-diabetic agents, medicinal plants continue to play very vital role in the management of diabetes mellitus in developing country. It has been reported that plants products that are effective against diabetic disease are cost effective and less toxic than synthesised drugs (Jung et al., 2006; Patel et al., 2012). In view of World Health Organization (WHO) recommendation on the use of medicinal plants for the management of diabetes mellitus, there is need to expand the frontiers of scientific research to discover more plants with anti-diabetic properties, especially now that recent findings have shown a rise in number of new cases of type 2 DM with an earlier onset and associated complications in developing countries. Hence upturning the earlier believe that diabetes is mainly found in developed countries of the world.

Through *in vivo* studies using animal models, a number of plants with anti-diabetic activity have been discovered (Bnouham et al., 2006; Kayarohanam et al., 2015; Mussie et al., 2015; Tsabang et al., 2017), but there is paucity of studies on *V. major* in relation to diabetic activity and other biochemical parameters. The present study investigated the effect of ethanolic leaf extract of *Vinca major* L. on biochemical parameters and glucose level of alloxan induced diabetic rats.

## MATERIALS AND METHODS

### Plant collection, identification and preparation

Fresh leaves of *V. major* L. were collected from the plant between the months of February and March 2018, in Choba, Rivers State, Nigeria. Taxonomic identification was done and voucher specimen was deposited at the Herbarium unit of Rhema University, Nigeria. The identified leaves were air dried for two weeks and then blended into powder.

### Plant extraction and toxicity study

The method as described by Adebayo et al., (2006) was used for preparation of plant extract. The powdered leaves of *V. major* (800 g) were soaked in 8 L of 70% ethanol for four days, after which the extract was filtered using a Whatman no. 1 filter paper and a cotton

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wool. It was further concentrated at 50°C using a rotary evaporator and further concentrated using water bath at 48°C. The weight of the extract obtained was 80 g giving a percentage yield of 10%. The toxicity study (LD<sub>50</sub>) was carried using the method as described by Lorke (1983), and the toxic dosage (LD<sub>50</sub>) for the leaf extract was found to be above 6,000 mg/kg.

### Experimental design, induction of diabetes and treatment of experimental animals

A total of sixty four male Wistar albino rats were procured from the animal colony of Department of Biochemistry, University of Port Harcourt, Nigeria for the present study. The rats were housed in plastic cages covered with wire mesh with facilities for food and water in them. The water and feeds were given *ad libitum*. The rat feed was a brand of commercial grower freshly obtained from a feed dealer along Abayi road, Aba, Nigeria. After acclimatization period, the experimental rats were made diabetic by Induction with alloxan monohydrate which was prepared by dissolving 10 g in 100 mL sterilized water. By single intraperitoneal injection, diabetes was induced in fasting rats by administration of 150 mg/kg body weight alloxan to each rat. Rats with blood glucose level above 200 mg/dL (after three days of post induction) were considered diabetic and chosen for the study.

The induced diabetic rats were then separated into four (1-4) groups of sixteen rats each. Group 1 served as the control, while groups 2 to 4 served as test groups. The treatments given to the groups are shown below

Group 1(Control): Diabetic rats treated normal saline (0.9% (w/v) NaCl)

Group 2: Diabetic rats treated with 100 mg/kg body weight of extract

Group 3: Diabetic rats treated with 250 mg/kg body weight of the extract

Group 4: Diabetic rats treated with 450 mg/kg body weight of the extract

The extract was given orally daily. The groups were placed on the same feed and water for twenty-eight days. The floors of the cages were constantly cleaned at interval of two days, their feed consumption and body weights were taken at interval of seven days.

Experimental handling of animals was in accordance with international guidelines on animal care and uses (NIH, 1985).

### Samples collection for analysis

Four rats from each group were sacrificed weekly (7, 14, 21 and 28 days) after subjecting them to overnight fast. The rats were subsequently anaesthetized with diethyl-ether and blood samples were collected by cardiac puncture into clean tubes for liver enzymes, electrolyte ions, urea, creatinine and glucose studies. Blood samples for haematological indices were collected with anticoagulant tubes. The tubes were properly labelled and used for analysis.

### Haematology analysis

The autoanalyzer machine was used for hematological analysis. The blood samples contained in the anticoagulant tubes were swirled/rolled on the blood roller each for five seconds and then opened and put under the probe of the autoanalyzer. The probe then collected the blood from the tubes for about 10 s and entered back into the haematology machine. The result was then printed a few seconds later, giving the parameters haemoglobin (Hb), red

blood cells (RBC), white blood cells (WBC), lymphocytes, monocytes, Basophils, mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC). Haematocrit (PCV) level was estimated using microhaematocrit methods as described by Alexander and Griffiths (1993).

### Biochemical assays

All the biochemical parameters investigated were measured using BUCK 211 spectrophotometer, England. The following biochemical parameters were carried out in serum; urea was analysed using the Bethlot Searcy's method (Searcy et al., 1967); creatinine was determined by the method described by Larsen (1971). Sodium ion (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ion levels were determined according to the instructions on their diagnostic kits purchased from Randox laboratories (UK). Potassium ion (K<sup>+</sup>) was determined by direct spectrophotometric method. Bicarbonate (HCO<sub>3</sub><sup>-</sup>) was determined using Forrester et al., (1979) method. Urea estimation was done using Urease-Berthlot method. Creatinine was estimated using the method described by Heinegård and Tiderström (1973). Total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol), and triglyceride lipid profiles were assayed enzymatically with their test kits (Randox Laboratories, England). The relations as described by Friedwald et al. (1972) were used for low density lipoprotein cholesterol (LDL-cholesterol) estimation. Liver enzyme such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using Reitman and Frankel (1957) methods; alkaline phosphatase (ALP) was carried out using the phenolphthalein monophosphate method (Babson et al., 1966); total bilirubin was calculated using Doumas et al., (1973) method; whereas glucose level was obtained using the enzymatic GOD-PAP method as described by Trinder (1969).

### Statistical analysis

Results are presented as mean and standard deviations of triplicate determinations. Group comparisons were done using least significant difference (LSD). Significant difference was established at 5% level as described by Onu and Igwemma (2000). Bars of the same day with different letters of alphabet are statistically significant at p<0.05.

## RESULTS

Figures 1 to 9 show haematology result of rats given *V. major* leaf extract at different concentrations. From the Figures, Hb ranged from 9.11 to 12.85 g/dl (Figure 1); haematocrit or PCV ranged from 25.80 to 38.00% (Figure 2); RBC ranged from 2.75-5.48×10<sup>9</sup> cell/L (Figure 3); WBC ranged from 2.10-4.53×10<sup>9</sup> cell/L (Figure 4); lymphocytes ranged from 35.10-66.00% (Figure 5); monocytes ranged from 14.10-19.95% (Figure 6); basophil ranged from 1.50-4.68% (Figure 7); mean cell volume (MCV) ranged from 71.70-98.30 fl (Figure 8); and mean corpuscular haemoglobin concentration (MCHC) ranged from 26.60-36.58 pg/dl (Figure 9).

Results of electrolyte ions, urea and creatinine as presented in Figures 10 to 15 show that sodium ion (Na<sup>+</sup>) ranged from 71.00 to 107.55 mEq/L (Figure 10); potassium ion (K<sup>+</sup>) ranged from 4.11 to 6.36 mEq/L (Figure 11); Chloride ion (Cl<sup>-</sup>) ranged from 60.71 to 92.73 mEq/L (Figure 12), bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) ranged from

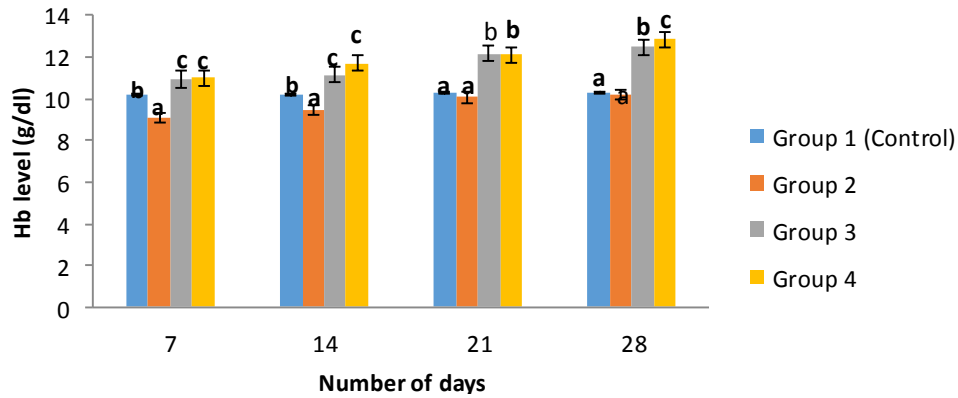


Figure 1. Hb level of the diabetic rats treated with *V. major* leaf extract for 28 days.

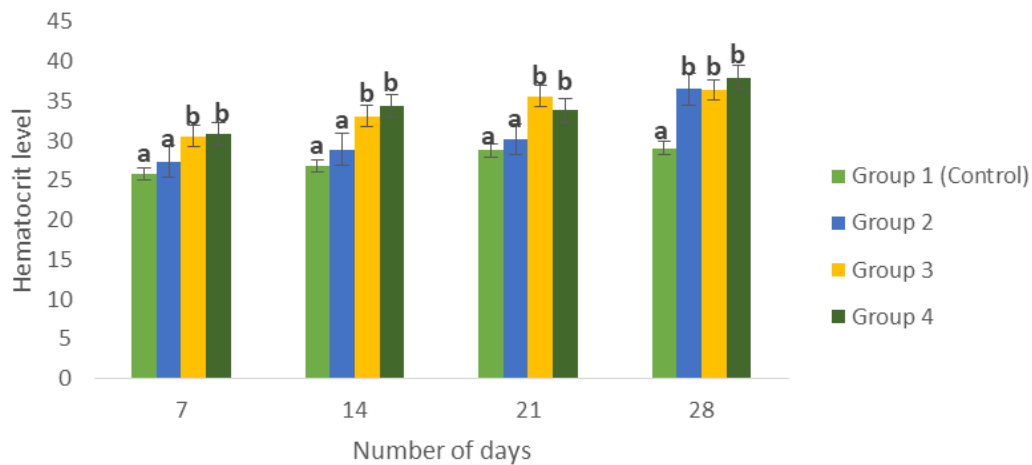


Figure 2. Haematocrit level of diabetic rats treated with *V. major* leaf extract for 28 days.

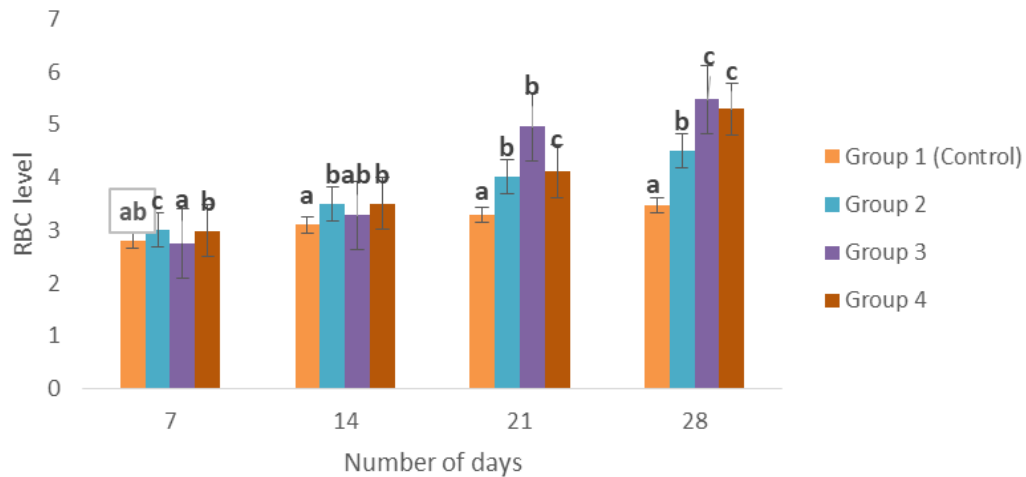
16.54 to 21.35 mmol/L (Figure 13); urea ranged from 38.12 to 56.56 mg/dL (Figure 14) and creatinine ranged from 0.01 to 4.36 mg/dL (Figure 15).

The lipid profiles as presented in Figures 16 to 19 reveal that total cholesterol ranged from 69.67 to 123.10 mg/dL (Figure 16), triglyceride ranged from 98.38 to 147.18 mg/dL (Figure 17); HDL cholesterol ranged from 16.34 to 24.01 mg/dL (Figure 18) and LDL cholesterol ranged from 30.60 to 83.53 mg/dL (Figure 19). Liver enzymes, total bilirubin, protein and glucose levels as presented in Figures 20 to 24 show that AST was between 16.17 to 27.15 U/L (Figure 20); ALT ranged 46.93 to 62.10 U/L (Figure 21); ALP ranged 12.71 to 25.31 U/L (Figure 22); total bilirubin ranged from 0.14 to 0.25 mg/dl (Figure 23); and glucose ranged from 224.76 to 306.37 mg/dl (Figure 24).

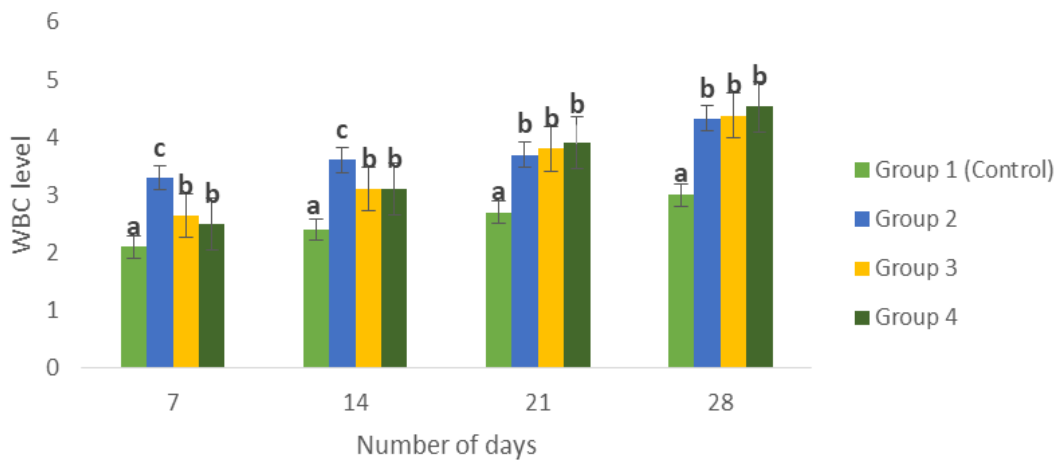
**DISCUSSION**

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds in the body (Mohammed et al., 2009; Duru et

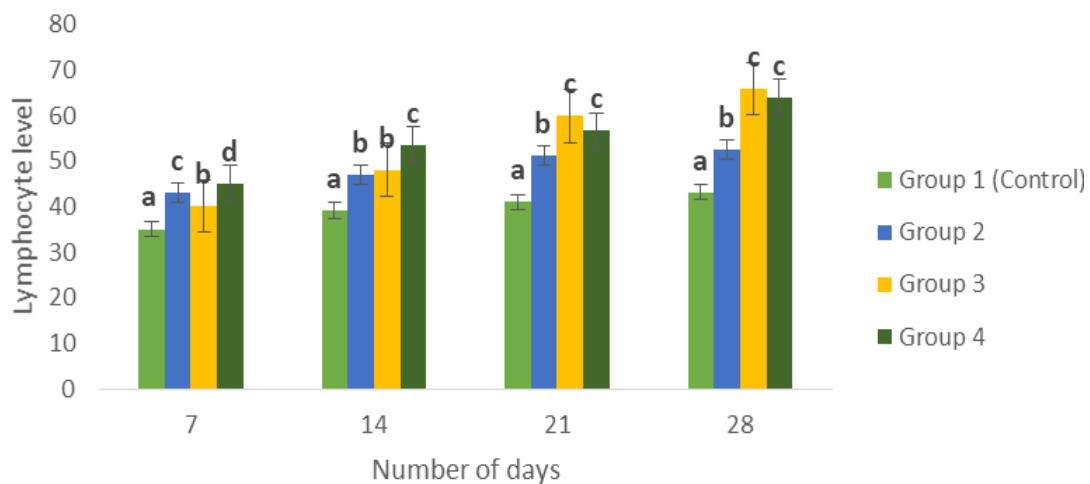
al., 2012b; Duru et al., 2018). Both plant extracts and free radical from alloxan, on the blood constituents of an animal are amongst the foreign compounds (Mohammed et al., 2009). According to Lebovitz (1994) and Andreoli et al., (1990), blood relating functions are amongst the aberration of diabetes mellitus. Haemoglobin (Hb) levels in groups 3 and 4 rats significantly increased ( $p < 0.05$ ) against the control throughout the number of days of the study. However, Hb in group 2 rats reduced significantly ( $p < 0.05$ ) when compared to the control (Group 1) on the 7<sup>th</sup> and 14<sup>th</sup> days, but increased insignificantly ( $p > 0.05$ ) against the control on the 21<sup>st</sup> and 28<sup>th</sup> days of the study (Figure 1). Sheela and Augusti (1992) noted that diabetic rats form glycosylated haemoglobin in results in decreased total haemoglobin. The increase in Hb levels of test rats in the present study could be attributed to the ability of *V. major* leaf extract to induce Hb production in diabetic condition. Haematocrit levels in rats of test groups 3 and 4 increased significantly ( $p < 0.05$ ) when compared to the control (Group 1). Haematocrit in group 2 increased insignificantly ( $p > 0.05$ ) in test group 2 rats against the control. However, the observed increase



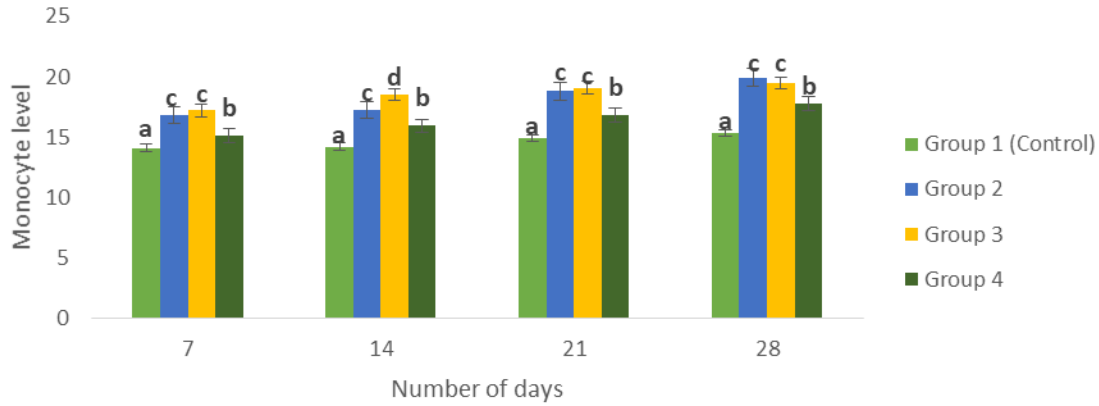
**Figure 3.** RBC level of diabetic rats treated with *V. major* leaf extract for 28 days.



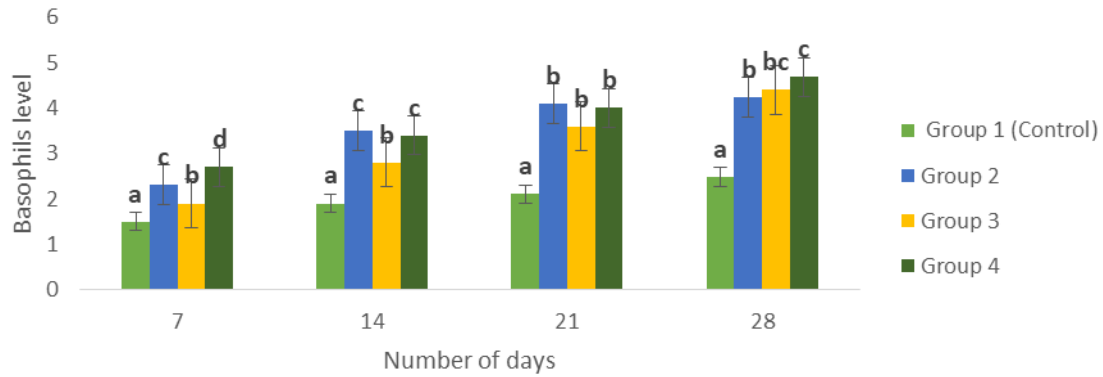
**Figure 4.** WBC level of diabetic rats treated with *V. major* leaf extract for 28 days.



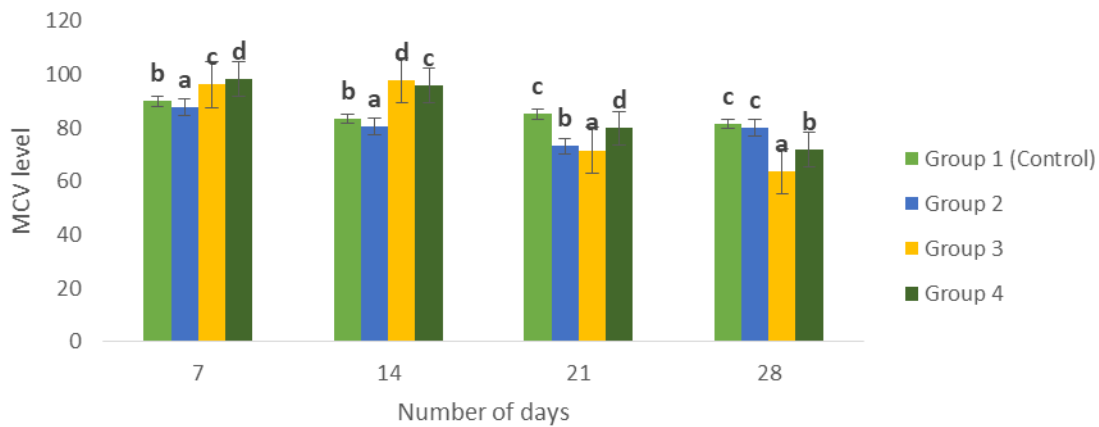
**Figure 5.** Lymphocyte level of diabetic rats treated with *V. major* leaf extract for 28 days.



**Figure 6.** Monocyte level of diabetic rats treated with *V. major* leaf extract for 28 days.



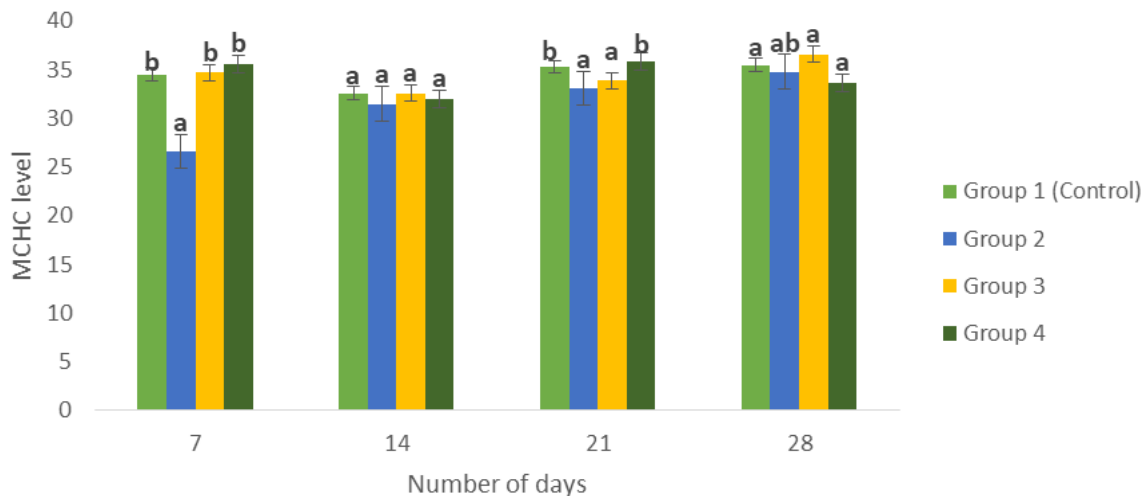
**Figure 7.** Basophils level of diabetic rats treated with *V. major* leaf extract for 28 days.



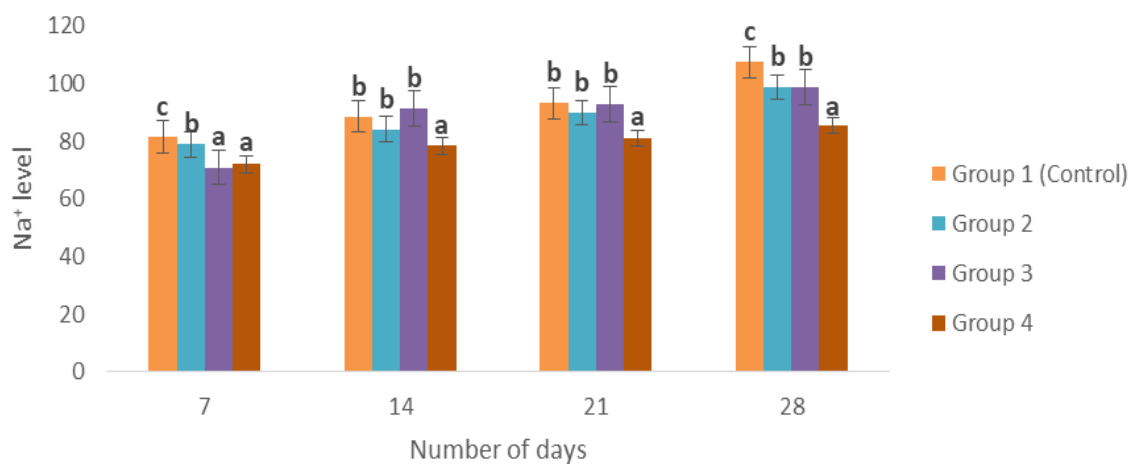
**Figure 8.** Mean cell volume (MCV) level of diabetic rats treated with *V. major* leaf extract for 28 days.

became significant ( $p < 0.05$ ) on the 28<sup>th</sup> day of the study (Figure 2). Saliu et al., (2012) attributed decrease in haematocrit in diabetic condition to cellular damage on the erythrocyte membrane as a result of oxidative stress

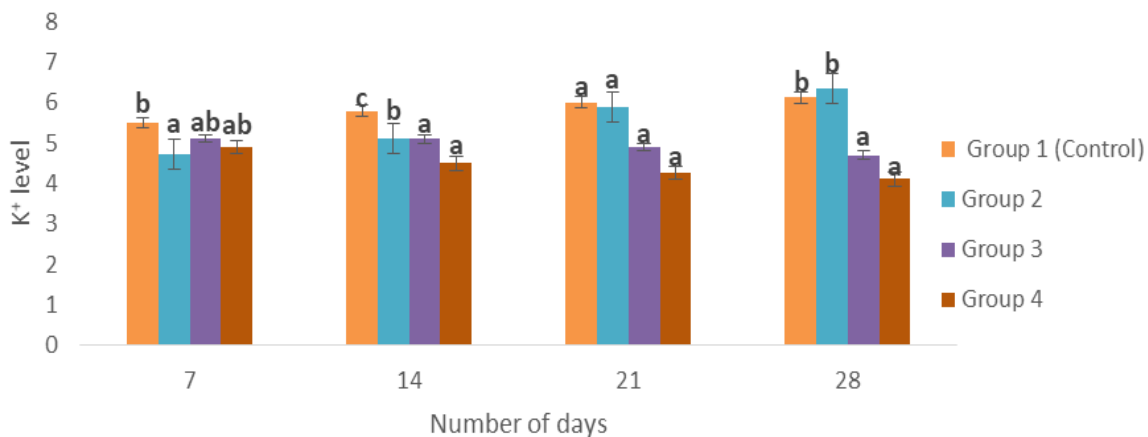
by agent of induction. Haematocrit measures the percentage by volume of packed red blood cells (RBCs) in a whole blood sample after centrifugation. Only group 2 rats had significantly increased ( $p < 0.05$ ) RBC level



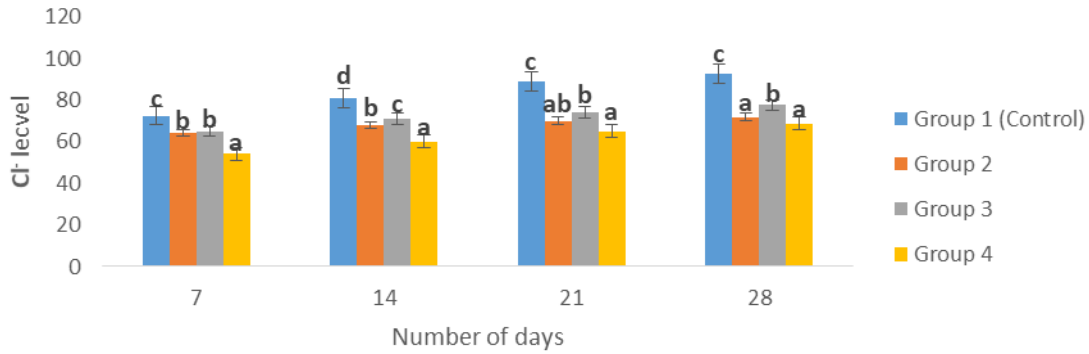
**Figure 9.** Mean corpuscular haemoglobin concentration (MCHC) level of diabetic rats treated *V. major* leaf extract for 28 days.



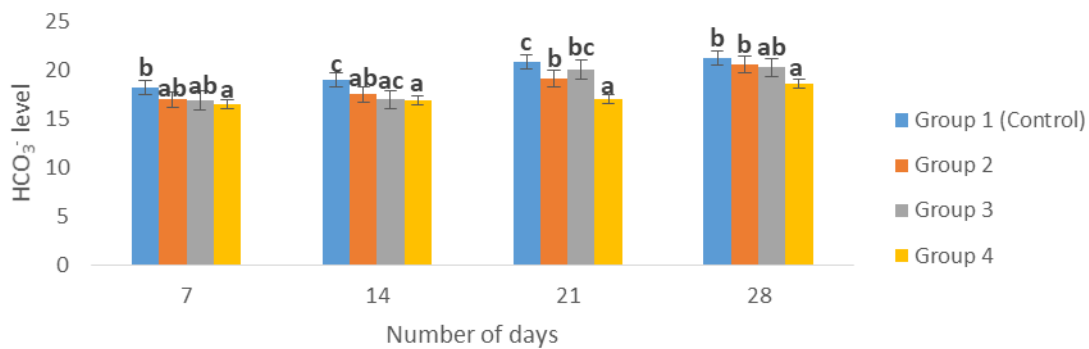
**Figure 10.**  $\text{Na}^+$  level of the rats treated with *V. major* leaf extract for 28 days.



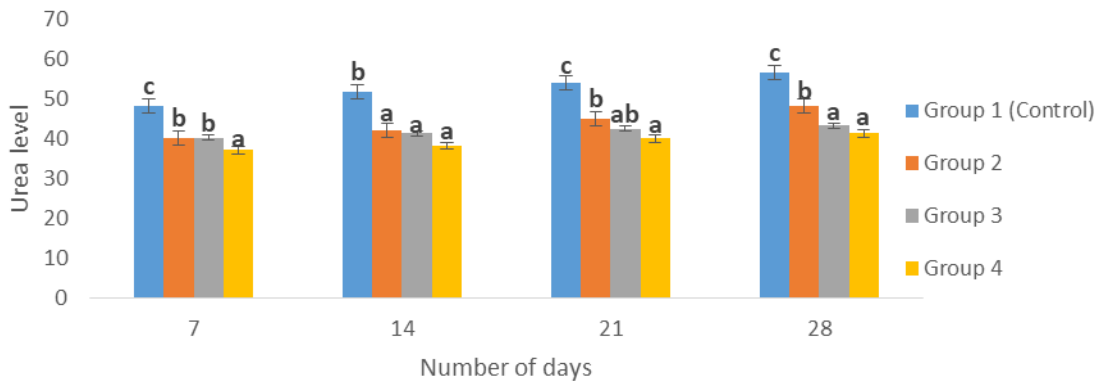
**Figure 11.**  $\text{K}^+$  level of diabetic rats treated with *V. major* leaf extract for 28 days.



**Figure 12.** Cl<sup>-</sup> level of diabetic rats treated with *V. major* leaf extract for 28 days.



**Figure 13.** HCO<sub>3</sub><sup>-</sup> (mmol/L) of the rats treated with *V. major* leaf extract for 28 days.

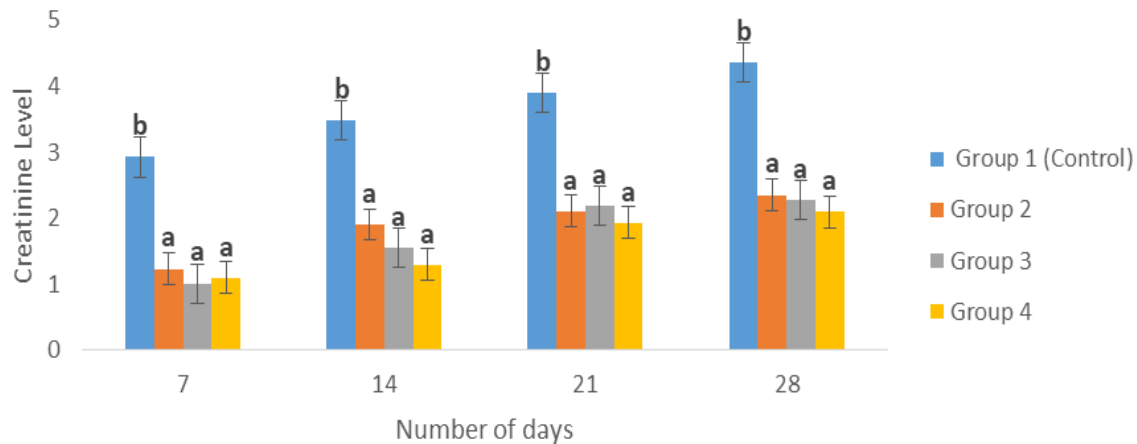


**Figure 14.** Urea level of diabetic rats treated with *V. major* leaf extract for 28 days.

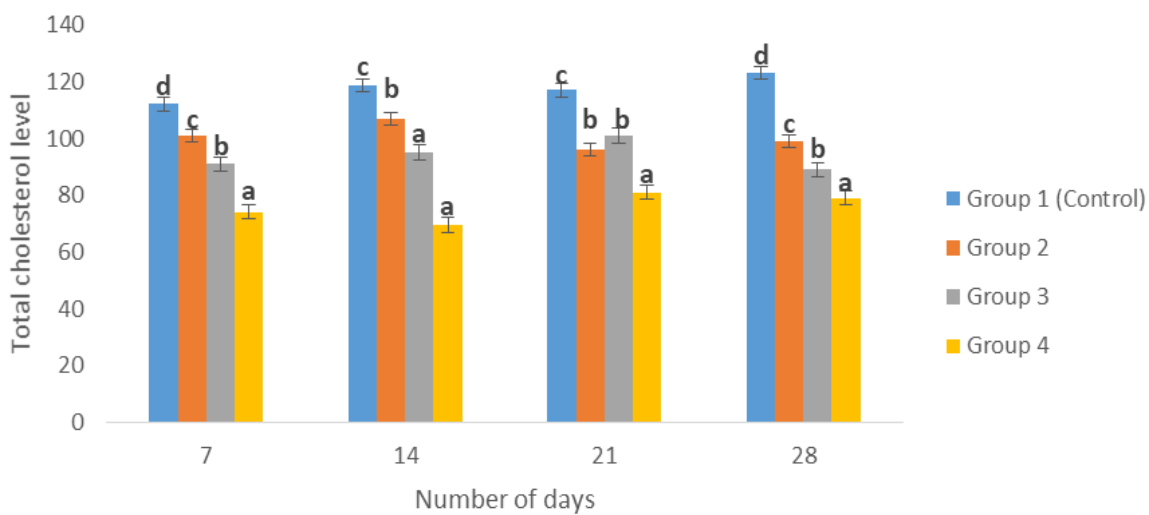
when compared to the control on the 7<sup>th</sup> day. RBC levels in groups 2 and 4 increased significantly ( $p < 0.05$ ) against the control on the 14<sup>th</sup> day, while all the test group rats had significantly increased ( $p < 0.05$ ) RBC levels on the 21<sup>st</sup> and 28<sup>th</sup> days of the study (Figure 3). According to Rao et al., (2003), reactive O<sub>2</sub> species generated during alloxan metabolism is implicated in red cell damage. The increase in RBC levels of test rats could give credence to the increased haematocrit, which may be linked to the

influence of *V. major* leaf extract on Hb production in diabetic condition. Edet et al., (2011) noted that alloxan diabetogenesis may cause perturbation in the bone marrow stem cells. This observation was experienced in the present study where the level of WBC in all the test rats significantly increased ( $p < 0.05$ ) when compared to the control throughout the study (Figure 4). White blood cell differentials are indicators of the ability of an organism to eliminate infection (Duru et al., 2012a, b;

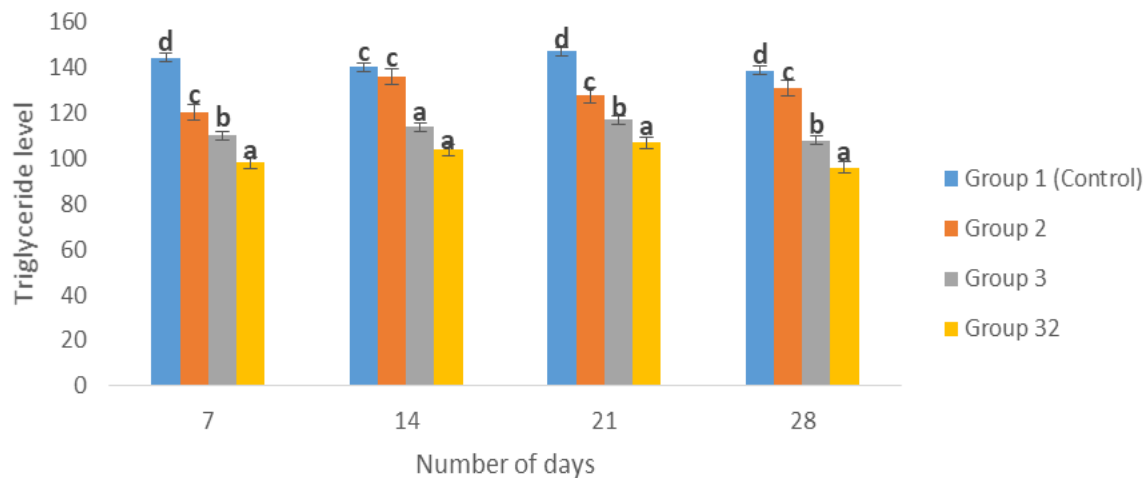




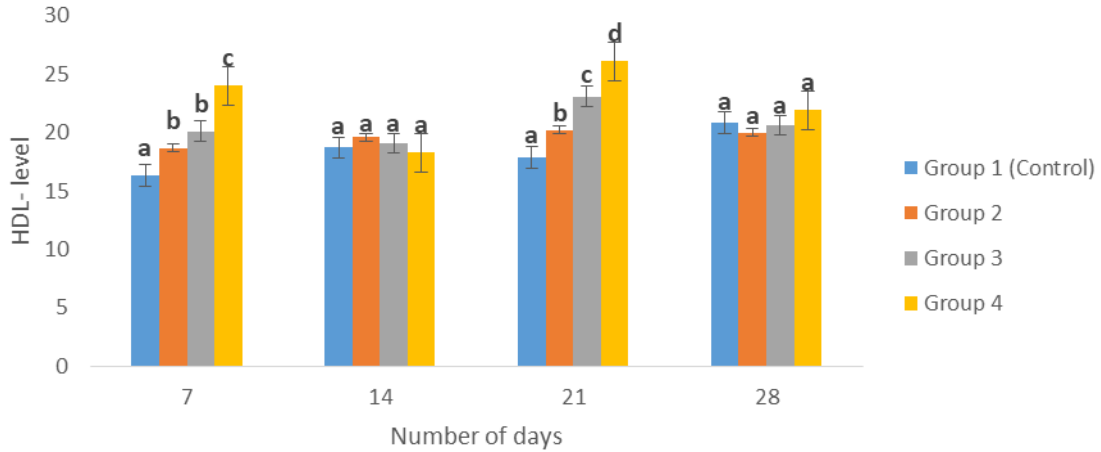
**Figure 15.** Creatinine level of diabetic rats treated with *V. major* leaf extract for 28 days.



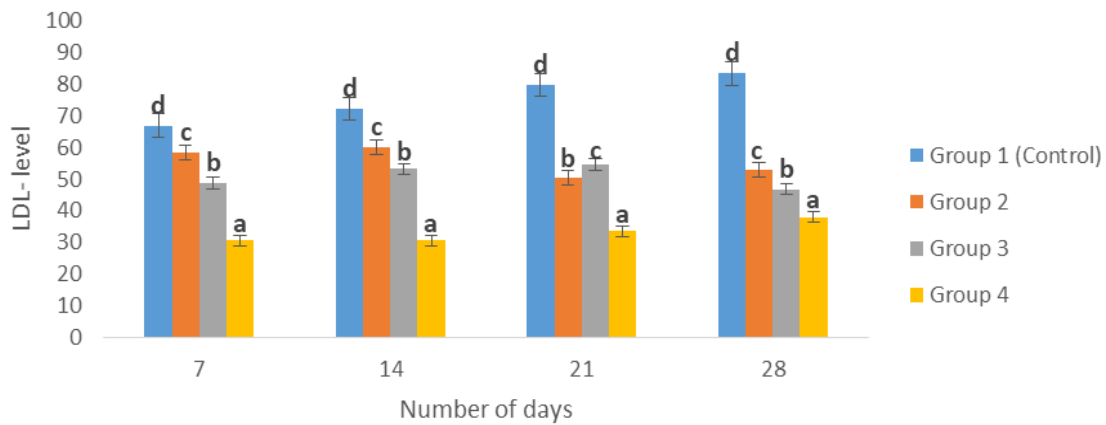
**Figure 16.** Total cholesterol level of diabetic rats treated with *V. major* leaf extract for 28 days.



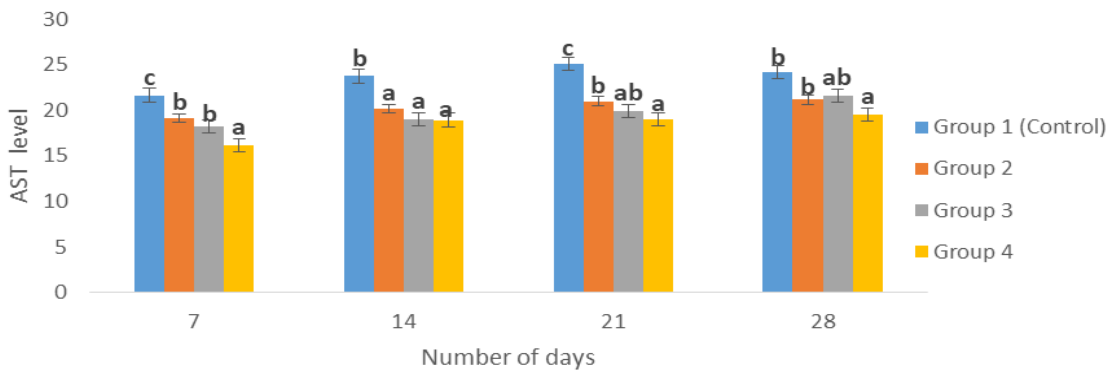
**Figure 17.** Triglyceride level of diabetic rats treated with *V. major* leaf extract for 28 days.



**Figure 18.** HDL-cholesterol level of diabetic rats treated with *V. major* leaf extract for 28 days.



**Figure 19.** LDL-cholesterol level of diabetic rats treated *V. major* leaf extract for 28 day.



**Figure 20.** AST level of diabetic rats treated with *V. major* leaf extract for 28 days.

Amadi et al., 2013; Ugboogu et al., 2016). Levels of lymphocytes, monocytes and basophils increased significantly ( $p < 0.05$ ) in test rats when compared to the

control (Figure 5 to 7). The observed increase in WBC differential could be indication of improved immune defence in test rats, and could be attributed to the

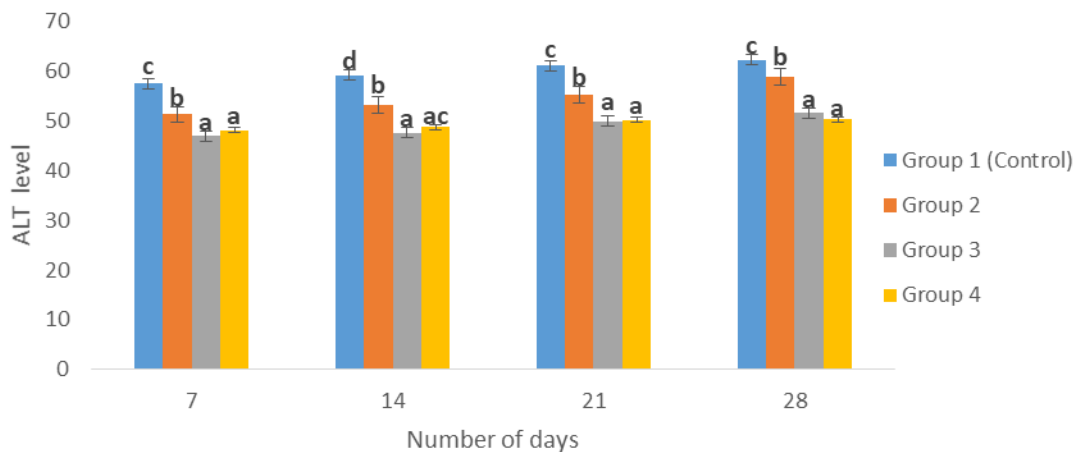


Figure 21. ALT level of diabetic rats treated with *V. major* leaf extract for 28 days.

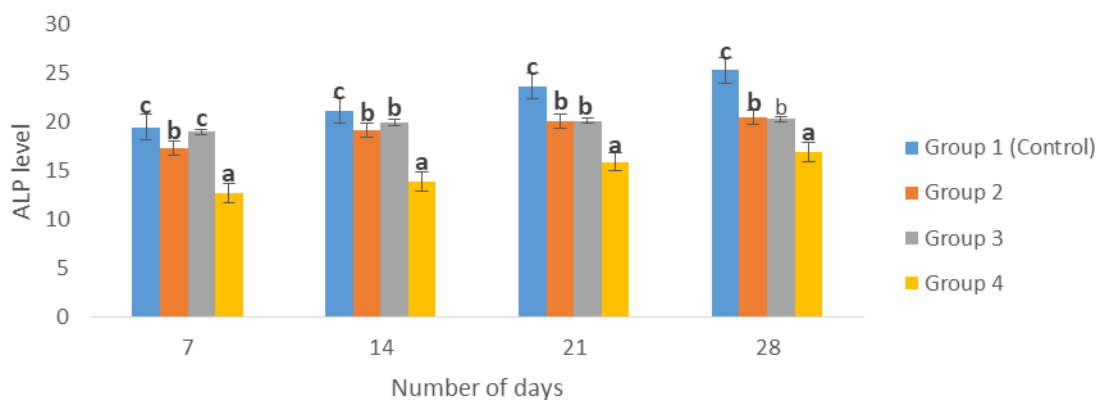


Figure 22. ALP level of diabetic rats after treated with *V. major* leaf extract for 28 days.

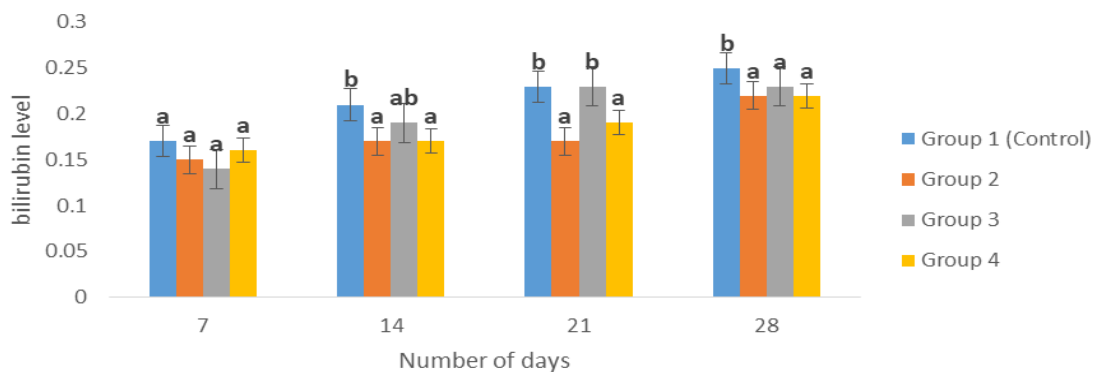
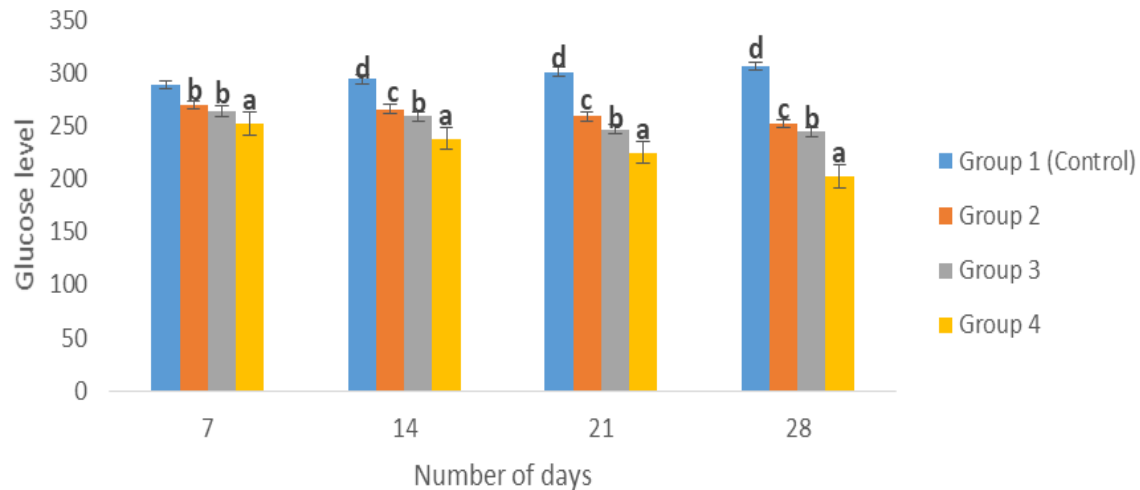


Figure 23. Total bilirubin level of diabetic rats treated with *V. major* leaf extract for 28 days.

presence *V. major* leaf extract. Mean cell volume (MCV) and Mean corpuscular hemoglobin concentration (MCHC) are related to individual red blood cells (Adebayo et al., 2005, 2010, Duru et al., 2018). MCV level of test

rats in groups 3 and 4 increased significantly ( $p < 0.05$ ) against the control on the 7<sup>th</sup> and 14<sup>th</sup> days. MCV of group 2 rats reduced significantly ( $p < 0.05$ ) when compared to the control on the 7<sup>th</sup> and 14<sup>th</sup> days



**Figure 24.** Glucose level of diabetic rats treated with *V. major* leaf extract for 28 days.

(Figure 8). The trend however reversed on the 21<sup>st</sup> and 28<sup>th</sup> days of the study. All the test rats had significantly reduced ( $p < 0.05$ ) MCV level on the 21<sup>st</sup> day while only test groups 3 and 4 rats reduced significantly ( $p < 0.05$ ) against the control on the 28<sup>th</sup> day (Figure 8). MCHC increased insignificantly ( $p > 0.05$ ) in rats test groups 3 and 4 on the 7<sup>th</sup> day against the control. MCHC was also insignificantly affected ( $p > 0.05$ ) in all the test rats against the control on the 14<sup>th</sup> and 28<sup>th</sup> days of the present study (Figure 9). It has been noted that several haematological changes affecting the red blood cells (RBCs), white blood cells (WBCs) and the coagulation factors are directly associated with diabetes mellitus (Wong and Lin, 1998; Bunza and Dallatu, 2017). Mansi and Lahham (2008) revealed that various hematological parameters and the immune system were reported to be altered during the course of diabetes. Hence, all the observed improvements on haematological parameters of the test rats against the control could be attributed to the treatment with *V. major* leaf extract.

Electrolyte ions, urea and creatinine are indices for evaluation of excretory organ (Duru et al., 2012b, 2013). Pecoits-Filho et al., (2016) noted that diabetic kidney disease is one of the most frequent and dangerous complications of diabetes mellitus. High level of blood sugar, genetics and blood pressure are among the factors that facilitate renal organ disease in diabetic condition.  $\text{Na}^+$  reduced significantly ( $p < 0.05$ ) in test rats against the control on the 7<sup>th</sup> and 28<sup>th</sup> days; while on the 14<sup>th</sup> and 21<sup>st</sup> days, only rats in test group 4 had insignificant reduction when compared to the control (Figure 10).  $\text{K}^+$  in test group 2 rats reduced significantly ( $p < 0.05$ ) against the control on the 7<sup>th</sup> day; all the test group rats had significantly reduced  $\text{K}^+$  when compared to the control on the 14<sup>th</sup> day (Figure 11); whereas only test groups 3 and 4 rats had significantly ( $p < 0.05$ ) reduced  $\text{K}^+$  against the control on the 21<sup>st</sup> and 28 days of

the study (Figure 11).  $\text{Cl}^-$  in the test rats reduced ( $p < 0.05$ ) significant against the control (Figure 12).  $\text{HCO}_3^-$  significantly reduced ( $p < 0.05$ ) in test group 4 rats on the 7<sup>th</sup> and 28 days against the control (Figure 13); those of test group 2 and 4 reduced significantly ( $p < 0.05$ ) on the 4<sup>th</sup> day against the control; on the 21<sup>st</sup> day, all the test rats had significantly reduced ( $p < 0.05$ )  $\text{HCO}_3^-$  when compared to the control (Figure 13). Urea and creatinine are both important parameters of renal function. The levels of urea and creatinine in test rats reduced significantly ( $p < 0.05$ ) against the control (Figures 14 and 15). The observed trend followed by electrolyte ions, urea and creatinine in test rats against the control in the present study, may be attributed to *V. major* leaf extract trying to salvage the excretory organs of alloxan battered diabetic rats.

Diabetic dyslipidaemia is a common experience in diabetic condition. It is normally the aetiology of premature coronary heart disease and atherosclerosis. There is increasing evidence that dyslipidaemia in diabetes is associated with increased risk of cardiovascular disease, which is the leading cause of death in patients with type 2 diabetes (Ronald, 2004). Levels of total cholesterol (Figure 16), triglyceride (Figure 17), and LDL-cholesterol (Figure 19) reduced ( $p < 0.05$ ) significantly in test rats when compared to the control for the number days of study. Cholesterol is needed by the body to maintain healthy cell status (Njoku et al., 2017). However, its high level in the body leads to coronary artery disease (Njoku et al., 2017). Triglyceride is a type of fat found in the blood and its high levels are related to higher risk of heart and blood vessel disease (Duru et al., 2014). Decrease in LDL-cholesterol, the bad cholesterol has been linked to reduced risk of coronary heart diseases (Glew, 2006; Shen, 2007; Duru et al., 2017); while increase in high levels of HDL-cholesterol are linked to a reduced risk of heart and blood vessel disease

(Shen, 2007). HDL-cholesterol of diabetic treated rats increased significantly ( $p < 0.05$ ) against the control on the 7<sup>th</sup> and 21 days of the study (Figure 18). The observations made on lipid profile parameters in this study could be indication of the salvage power of *V. major* leaf extract on diabetic dyslipidaemia.

Different authors have noted that hepatic dysfunction is among the abnormalities of diabetic condition (Yakhchalian et al., 2018). There are increasing evidence on occurrence of hepatic necrosis and inflammation induced by diabetes mellitus pathogenesis (West, 2000; Kyle et al., 2002). Inflammation and liver injuries that lead to damage of hepatocytes are also ascribed to agent of diabetic induction in experimental animals such as alloxan and streptozotocin (Jacobs et al., 2004; Zafar et al., 2009; Pagana and Pagana, 2013). AST, ALT and ALP liver enzymes reduced significantly ( $p < 0.05$ ) in test rats against the control (Figures 20 to 22). Total bilirubin reduced insignificantly ( $p > 0.05$ ) in test rats against the control on the 7<sup>th</sup> day, the observed reduction became significant ( $p < 0.05$ ) in test groups 2 and 4 rats on the 14<sup>th</sup> and 21<sup>st</sup> days whereas the significant reduction in total bilirubin manifested in all the test rats on the 28<sup>th</sup> day (Figure 23). Level of glucose in test rats reduced significantly ( $p < 0.05$ ) when compared to the control (Figure 24). Many studies have reported that single diabetogenic dose of alloxan or streptozotocin showed an increase in glucose, ALT and AST levels (Zafar et al., 2009, Zafar and Naqvi 2010; Ahmed et al., 2012). The observed reduction in AST, ALT, ALP and glucose in test rats in this could be attributed to the ability of *V. major* leaf extract to improve on the hepatic necrosis and inflammable associated with diabetic condition.

## Conclusion

Diabetic rats treated with *V. major* leaf extract in this study, showed significant improvements on those associate problems of diabetes which include anaemia, dyslipidaemia, and hepatic necrosis and inflammation. Rats treated with the leaf extract also showed reduced glucose level (Hypoglycemia). From the observations of this study, extract from leaf of *V. major* may be effective against diabetes and some of its associate problems. There is need to urgently extend the scope of the present study to accommodate the isolation of the active ingredients or compounds that could be responsible for these actions in *V. major* leaf.

## CONFLICT OF INTERESTS

The Authors declare no conflict of interests and are all aware that the article has been submitted to African Journal of Biotechnology. The Authors also declare that extra authors may be added as the case maybe.

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