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Effects of n-Hexane fraction of *Gongronema latifolium* on some biochemical parameters in alloxan-induced diabetic albino Wistar rats

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ABSTRACT

Diabetes mellitus is a burden affecting both developed and developing countries of the world and it has been known to be associated with hyperglycaemia, lipid disorders and cardiovascular complications. This work was undertaken to ascertain the effects of the n-Hexane leaf fraction of *Gongronema latifolium* as an anti-diabetic agent using standard methods. The results indicated a significant percentage increase ($p<0.05$) in the body weight of Groups 2 (33.14), 4 (12.55) and 5 (22.01) when compared to Group 3 (-34.02); there was a significant decrease ($p<0.05$) in the body weight of Group 3 when compared to Group 1 (10.12). The blood glucose results showed a significant percent decrease ($p<0.05$) in Groups 2 (66.76 ± 10.81), 4 (53.24 ± 5.61) and 5 (59.34 ± 11.41) when compared to Group 3 (9.91 ± 15.64) and a significant increase ($p<0.05$) in Group 3 when compared to Group 1 (3.78 ± 1.18). The liver enzyme AST (U/L) of Groups 4 (55.80 ± 1.36) and 5 (41.60 ± 2.01) increased significantly ($p<0.05$) when compared to Groups 1 (25.80 ± 7.55) and 2 (29.80 ± 2.75) and decreased significantly ($p<0.05$) when compared to Group 3 (69.80 ± 4.83). Groups 4 (44.40 ± 30.50) and 5 (53.40 ± 24.25) α-amylase levels (U/L) reduced significantly ($p<0.05$) when compared to Groups 1 (90.80 ± 31.08) and 2 (78.20 ± 21.15) and increased significantly ($p<0.05$) when compared to Group 3 (24.80 ± 17.84). Lipid profile (mmol/l) indicated a significant increase ($p<0.05$) in HDL-C of Groups 2 (1.83 ± 0.10), 4 (1.31 ± 0.11) and 5 (1.94 ± 0.15) when compared to Group 3 (0.30 ± 0.07) and a significant decrease ($p<0.05$) in HDL-C of Group 3 when compared to Group 1 (2.24 ± 0.22). TG, TC, LDL and VLDL showed a significant increase ($p<0.05$) in Group 3 when compared to Group 1 and a significant decrease ($p<0.05$) in Groups 2, 4 and 5 when compared to Group 3. Haematological parameters were also affected by the fraction. The results of this study suggest that the n-Hexane fraction of *G. latifolium* may possess hypoglycaemic and hypolipidaemic effects and seems to weaken the haematopoietic system.

Key words: Diabetes mellitus, *Gongronema latifolium*, alloxan, haematopoietic, hypoglycaemic, hypolipidaemic

INTRODUCTION

Diabetes mellitus is a non-communicable metabolic disorder characterized by hyperglycaemia due to overproduction and underutilization of glucose. It is a complex disorder caused by increased hepatic glucose

production, impaired insulin action and no insulin production leading to a rise in blood glucose level (Srinivasan et al., 2012; Das and Elbein, 2016). The generation of reactive oxygen species (ROS) appears to play a critical role in the pathogenesis of diabetes mellitus (Harnett et al., 2010). Hyperglycaemia associated with diabetes also increases the production of ROS and affects antioxidant enzymes and reactions (Kowluru et al*.,* 2010).

Plants are sources of potential therapeutic agents against various diseases due to their biodiversity and presence of a wide array of bioactive phytochemicals and secondary metabolites (Farombi, 2003). Amongst them is *Gongronema latifolium* whose individual and synergistic anti-diabetic effects have been reported (Atangwho et al*.*, 2010; Effiong et al., 2017). *G. latifolium* is a perennial edible shrub of the family, Asclepiadaceae widely employed in Nigeria for various medicinal and nutritional purposes (Morebise et al., 2002; Ugochukwu et al*.,* 2011)). Scientific studies have established the hypoglycaemic, hypolipidaemic, anti-inflammatory and antioxidative effects of the crude extract of *G. latifolium* leaf (Morebise et al*.,* 2002; Ugochukwu et al*.,* 2003).

Alloxan, an oxygenated pyrimidine derivative is a toxic glucose analogue, which selectively destroys insulin producing beta cells in the pancreas when administered to rodents and many other animal species (Lenzen, 2008). This causes an insulin-dependent diabetes mellitus with characteristics similar to type 1 diabetes in humans. Hence, alloxan-induced diabetes in rats is a good experimental model to study diabetes (Szkudelski, 2001).

Lipid profile (or lipid panel) is the measurement of various lipids that are found in the blood. It reports lipid concentrations (Dashti and Wolfbauer, 2014). A lipid profile contains information about several different kinds of lipids that normally circulate in the blood. Lipids generally included in a blood lipid profile are total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides. An extended lipid profile may include very low-density lipoprotein (VLDL) (Priyadharshini and Thomas, 2011).

Enzyme activity changes is one of the characteristics of diabetes mellitus. Some enzymes that show changed activities in diabetes mellitus include: N-acetyl-β-D-glucosaminidase (NAG), acid phosphatase, trehalase, α-amylase and amino transferases (Beltiore et al*.,* 2013). However, α-amylase and the amino transferases are the enzymes that were considered in the course of this work.

There has been an increasing demand for the use of plant products with anti-diabetic activity due to low cost, easy availability and lesser side effects; and with various reports on the anti-diabetic activity of the crude leaf extracts of *G. latifolium* on diabetic rats, the present research work was to investigate the effect of the n-Hexane leaf fraction of *G. latifolium* in alloxan-induced diabetic rats to further ascertain its anti-diabetic property.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh but matured *G. latifolium* leaves were purchased from a local market at Abak Local Government Area of Akwa-Ibom State, Nigeria in June, 2017. The plant was identified and authenticated by Dr. (Mrs.) Uduak Eshiet of the Department of Botany, University of Uyo, Uyo, Nigeria. It was deposited at the Faculty of Pharmacy Hebarium with a voucher number UUPH9(a).

Extraction and fractionation of plant material

The crude plant extract was prepared using the wet method of extraction. A kilogram of the fresh leaves already washed and rinsed properly were chopped into pieces, blended using an electric blender in 1.5 L of ethanol, transferred into an amber colored bottle and kept for 72 h under 4°C in a dark compartment. On the third day, the solution was filtered first with a cheese cloth and then with Whatman No. 1 filter paper. Extract obtained was concentrated in vacuo with a rotary evaporator at 37-40°C and a desiccator containing a self-indicator silica gel was used to dry it completely. Fractionation was done using the liquid/liquid partitioning method and the solid ingredient was dissolved with 20 ml distilled water in a beaker. 100 ml of n-Hexane reagent was added; thereafter the mixture was poured into a separating funnel (500-ml) and shaken vigorously. It was allowed to stand for about 4 h for maximum extraction followed by evaporation of the liquid to dryness (at room temperature) leaving behind the n-Hexane fraction.

Experimental animals

A total of forty male albino Wistar rats of 150-180 g were used in the experimental analysis. The animals were obtained from the Basic Medical Sciences Faculty Farm House, University of Uyo, Nigeria and were later moved to the Animal House in the Department of Pharmacology and Toxicology, University of Uyo, Uyo, Nigeria. They were housed in clean cages of wooden bottom and wire mesh top. The animals were maintained under standard laboratory condition of humidity (50±5%) and temperature (28±2°C), and maintained in a 12-h light/dark cycle. The handling of the animals was approved by the Animal Ethics Committee of University of Uyo, Uyo, Nigeria. The animals had free access to feeds and water on a daily basis and were acclimatized for 14 days before the commencement of the experimental research.

Induction of diabetes

Diabetes was induced by single intraperitorieal injection of freshly prepared solution of alloxan monohydrate (150 mg/kg Body weight)

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Table 1. Experimental design.

Groups	No. of animals	Induction	Treatment	Dosage	Duration (Days)
I (Normal Control)	5	Non-induced	Distilled water	2 ml	14
II (Positive Control)	5	Diabetic	Glibenclamide	5 mg/kg	14
III (Negative Control)	5	Diabetic	Distilled water	2 ml	14
IV (Fraction of *G.I*)	5	Diabetic	n-Hexane fraction	300 mg/kg	14
V (Fraction of *G.I*)	5	Diabetic	n-Hexane fraction	400 mg/kg	14

**G.I = G. latifolium.*

Table 2. Result of administration of n-Hexane extract of *G. latifolium* on body weight of test and control groups.

Groups	Initial weight (g)	Final weight (g)	Percent change
Group I (Normal control)	133.33 ± 3.81	143.66 ± 6.14	10.12
Group II (Positive control)	123.13 ± 3.80	156.6 ± 5.34	33.14
Group III (Negative control	138.76 ± 5.08	104.33 ± 4.05	-34.02
Group IV (300 mg/kg)	127.73 ± 4.17[a,c]	139.33 ± 6.25[a,c]	12.55
Group V (400 mg/kg)	123.3 ± 3.05[b,c]	145.33 ± 4.51[a,b]	22.01

[a]Significantly different when compared with Group I at $p<0.05$; [b]Significantly different when compared with Group II at $p<0.05$; [c]Significantly different when compared with Group III at $p<0.05$.

dissolved in 0.9% saline (NaCl solution) in overnight fasted Wistar rats. The animals were placed on dextrose saline for two days to prevent hypoglycaemia while being kept for 72 h. Diabetes was assessed in the rats by determining the blood glucose concentration 3 days after injection of alloxan. The rats with blood glucose level above 200 mg/dl were selected for the study.

Experimental groups

A total of forty rats were divided (based on their blood glucose levels) into five groups of five rats each. Groups I, II and III served as normal, positive and negative controls and was given distilled water (2 ml), glibenclamide (5 mg/kg body weight) and distilled water (2 ml) respectively. Groups IV and V were administered the n-Hexane fraction of *G. latifolium* at 300 and 400 mg/kg of body weight respectively. Of the 5 groups, Groups II – V were alloxan-induced diabetic rats while Group I was non-induced diabetic rats. The administrations were through oral routes for 14 days. The experimental design is as shown in Table 1.

Biochemical assays

The kits used for the assay were obtained from Randox Laboratory Ltd., Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom. The method of Reitman and Frankel (1956) was used to determine the lipid profile levels and that of enzyme concentrations. The change in fasting blood glucose (FBG) levels was employed with the "tail-tipping" method and the glucometer used was the 'fine test' glucometer.

Statistical analysis

Results were analyzed with one-way ANOVA using SPSS followed by Kurkey-Kramer multiple comparison test. All data were expressed as Mean ± SEM and values of $P<0.05$ were considered significant.

RESULTS

Effects of n-Hexane fraction of *G. latifolium* on body weight of control and test groups

There was a significant increase ($p<0.05$) in body weights of Groups 1 (10.12%), 2 (33.14%), 4 (12.55%) and 5 (22.01%) after 14 days of treatment when compared to Group 3 (-34.02%) as shown in Table 2.

Effects of n-Hexane fraction of *G. latifolium* on blood glucose levels of control and test groups

The result shows that the effect of n-Hexane fraction of *G. latifolium* on blood glucose levels of treated diabetic rats indicated a significant decrease ($p<0.05$) in the final blood glucose levels of Groups 2, 4 and 5 when compared to that of Group 3. These were comparable to that of Group 1 as shown in Table 3 and Figure 1.

Effect of n-Hexane fraction of *G. latifolium* on liver and pancreatic enzymes

Table 4 and Figure 2 gives the effects of treatment on liver enzymes and serum amylase levels of test and control groups. The AST levels of Groups 4 and 5

Table 3. Result of administration of n-Hexane extract of *G. latifolium* on blood glucose levels of test and control groups.

Groups	Initial glucose (mg/dl)	Final glucose (mg/dl)	Percent change
Group I (Normal control)	81.40 ± 2.30	78.32 ± 1.18	3.78 ± 1.18
Group II (Positive control)	399.45 ± 0.09	132.76 ± 10.81	66.76 ± 10.81
Group III (Negative control)	303.61 ± 1.72	273.53 ± 15.64	9.91 ± 15.64
Group IV (300 mg/kg)	352.56 ± 2.05[c]	164.87 ± 5.61[c]	53.24 ± 5.61[c]
Group V (400 mg/kg)	349.50 ± 1.44[c]	142.09 ± 11.41[c]	59.34 ± 11.41[c]

[a]Significantly different when compared with Group I at p<0.05; [b]Significantly different when compared with Group II at p<0.05; [c]Significantly different when compared with Group III at p<0.05.

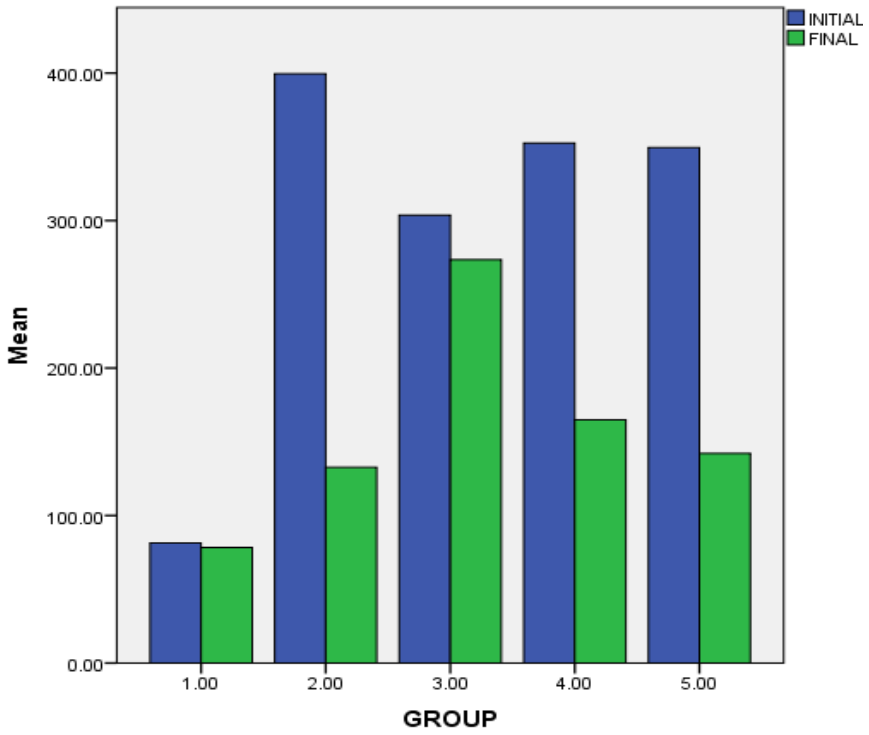

Figure 1. Graph of initial and final fasting blood glucose concentration.

Table 4. Result of administration of n-Hexane extract of *G. latifolium* on liver enzymes and serum amylase of test and control groups.

Groups	ALT (U/L	ALP (U/L)	AST (U/L)	α- Amylase (U/L)
Group I (Normal control)	19.40 ± 1.29	52.00 ± 2.74	25.80 ± 7.55	90.80 ± 31.08
Group II (Positive control)	21.80 ± 2.35	58.80 ± 1.71	29.80 ± 2.75	78.20 ± 21.15
Group III (Negative control)	39.60 ± 3.70	98.20 ± 0.73	69.80 ± 4.83	24.80 ± 17.84
Group IV (300 mg/kg)	27.00 ± 0.89	69.60 ± 3.94[c]	55.80 ± 1.36[a,b]	44.40 ± 30.50[a,b]
Group V (400 mg/kg)	22.60 ± 2.42	66.80 ± 7.01[c]	41.60 ± 2.01[a,b,c]	53.40 ± 24.25[a,b,c]

[a]Significantly different when compared with Group I at p<0.05; [b]Significantly different when compared with Group II at p<0.05; [c]Significantly different when compared with Group III at p<0.05.

increased significantly (p<0.05) when compared to Groups 1 and 2, while it decreased significantly (p<0.05) when compared to Group 3. Alpha amylase levels however decreased significantly (p<0.05) in Groups 4

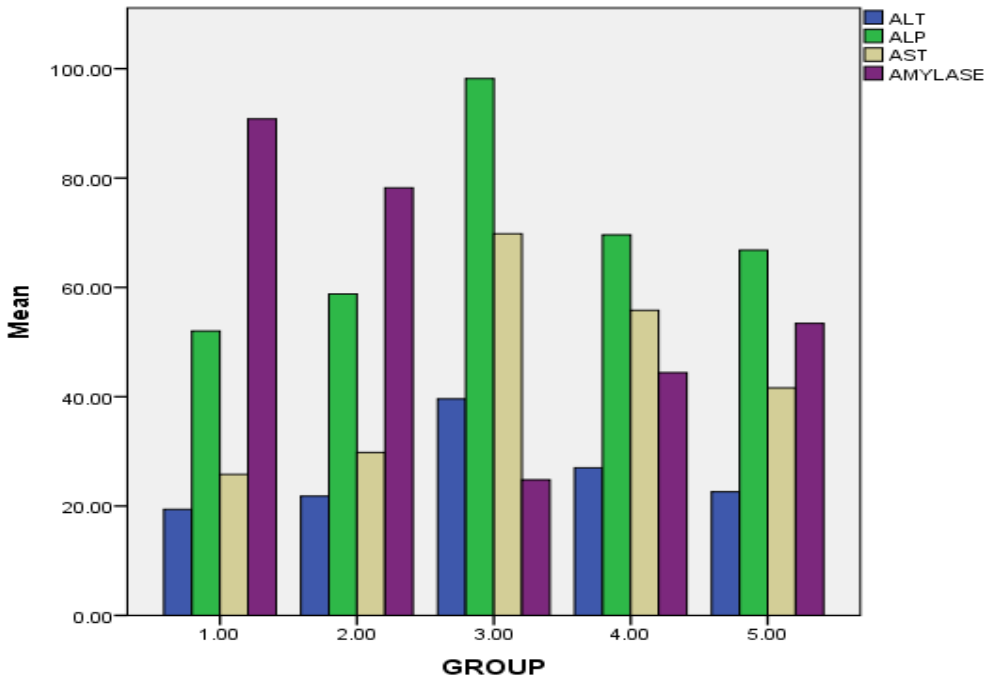

Figure 2. Graph of liver enzymes (ALT, ALP, AST) and serum amylase.

Table 5. Result of administration of n-Hexane extract of *G. latifolium* on lipid profile levels of test and control groups.

Groups	Total Cholesterol (mmol/l)	Triglyceride (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)
Group I (Normal control)	3.58 ± 0.02	1.44 ± 0.09	2.24 ± 0.22	2.6 ± 0.04	0.36 ± 0.10
Group II (Positive control)	3.24 ± 0.09	1.70 ± 0.07	1.83 ± 0.10	2.82 ± 0.09	0.42 ± 0.08
Group III (Negative control)	6.1 ± 0.16	3.34 ± 0.09	0.30 ± 0.07	4.9 ± 0.19	0.86 ± 0.05
Group IV (300 mg/kg)	3.92 ± 0.13[c]	1.94 ± 0.08[c]	1.31 ± 0.11[a, c]	2.94 ± 0.10[c]	0.48 ± 0.14[c]
Group V (400 mg/kg)	3.68 ± 0.24[c]	1.60 ± 0.24[c]	1.94 ± 0.15[a, c]	2.66 ± 0.08[c]	0.40 ± 0.11[a, c]

[a]Significantly different when compared with Group I at p<0.05; [b]Significantly different when compared with Group II at p<0.05; [c]Significantly different when compared with Group III at p<0.05.

and 5 when compared with Groups 1 and 2; whereas it significantly increased (p<0.05) when compared to group 3.

Effect of n-Hexane fraction of *G. latifolium* on lipid profile levels of control and test groups

The results shown in Table 5 and Figure 3 indicates a significant decrease (p<0.05) in the cholesterol concentration of Groups 4 and 5 when compared to Group 3. There was a significant decrease (p<0.05) in triglyceride levels of Groups 4 and 5 when compared against Group 3. The HDL levels of Groups 4 and 5 were significantly decreased (p<0.05) in comparison to that of Group 1 and significantly increased (p<0.05) when compared to Group 3. LDL and VLDL levels were significantly decreased (p<0.05) in Groups 4 and 5 when compared to Group 3.

Effect of n-Hexane fraction of *G. latifolium* on haematological indices of control and test groups

The result indicates a significant increase (P<0.05) in the WBC (white blood cell) count of Groups 4 and 5 when compared to Group 1 and a significant decrease (P<0.05) when compared to Group 3. There was a significant decrease (P<0.05) in neutrophil count of Group 4 and 5 when compared to Group 3 and a significant increase (P<0.05) in lymphocyte count of Groups 4 and 5 when compared to Group 1. There was a significant increase

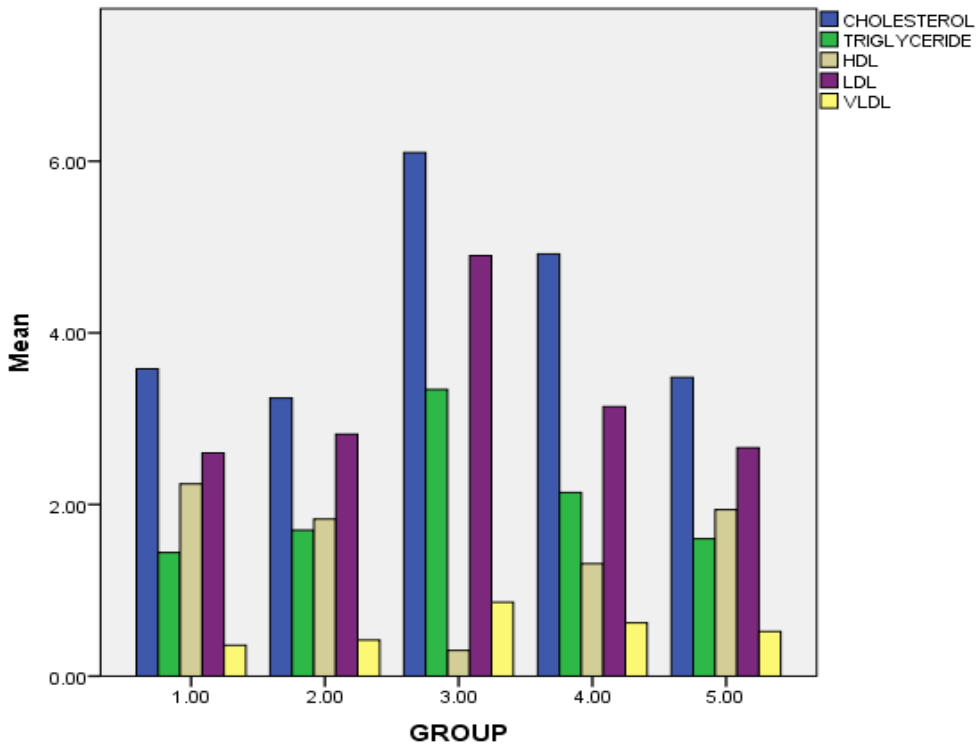

Figure 3. Graph of lipid profile levels (Cholesterol, Triglyceride, HDL, LDL, VLDL).

Table 6. Result of administration of n-Hexane extract of *G. latifolium* on haematological indices of test and control groups.

Groups	Group I	Group II	Group III	Group IV	Group V
WBC (×10^9/L)	7.05 ± 0.61	10.95 ± 0.68	25.14 ± 0.39	17.16 ± 0.42$^{a, b,c}$	13.74 ± 0.98^{a,c}
Neutrophils (%)	56.12 ± 1.35	64.18 ± 1.72	90.56 ± 5.22	79.88 ± 1.67^{c}	74.72 ± 1.52^{c}
Lymphocyte (%)	31.10 ± 1.17	34.20 ± 1.72	59.16 ± 5.19	48.06 ± 2.04^{a}	44.94 ± 1.02^{a}
Monocyte (%)	5.68 ± 0.15	7.80 ± 0.13	22.54 ± 0.30	18.34 ± 0.05^{a,b,c}	14.66 ± 0.58^{a,b,c}
Eosinophils (%)	3.28 ± 0.04	6.10 ± 0.25	10.40 ± 0.14	7.60 ± 0.08^{a,c}	5.28 ± 0.17^{a,c}
Basophils (%)	1.72 ± 0.06	3.10±0.07	6.90 ± 0.32	3.11 ± 0.09^{a,b,c}	3.82 ± 0.16^{a,b,c}
RBC (×10^{12}/L)	4.20 ± 0.18	4.64 ± 0.27	2.82 ± 0.30	3.17 ± 0.26^{a,b}	3.85 ± 0.23^{a,b}
HGB (g/dL)	12.74 ± 0.26	12.40 ± 0.35	7.00 ± 0.58	8.58 ± 0.44^{a,b}	9.36 ± 0.85^{a,b}
HCT (%)	38.60 ± 0.92	42.92 ± 0.86	23.20 ± 2.15	34.52 ± 1.30^{c}	44.94 ± 1.05^{c}
MCV(fL)	82.14 ± 0.94	72.32 ± 1.19	64.42 ± 1.19	75.42 ± 1.51^{c}	85.98 ± 1.07^{c}
MCH (pg)	28.76 ± 0.24	27.24 ± 1.39	18.16 ± 0.27	24.98 ± 0.54	28.70 ± 0.35
MCHC (g/dL)	33.30 ± 0.23	32.26 ± 0.31	22.60 ± 0.35	30.10 ± 0.72^{c}	36.44 ± 0.62^{c}
Platelet (×10^9/L)	123.20 ± 4.21	143.60 ±46.56	990.80 ± 26.23	401.80 ±37.35^{a,b,c}	364.00 ±33.93^{a,b,c}
MPV (fL)	6.72 ± 0.19	6.52 ± 0.21	17.16 ± 0.09	13.90 ± 0.27^{a,b,c}	12.22 ± 0.12^{a,b,c}
PCT	1.66 ± 0.40	2.6 ± 0.08	4.25 ± 0.24	3.58 ± 0.24	2.95 ± 0.30

(P<0.05) in monocyte count of Groups 4 and 5 when compared to Groups 1 and 2, along with a significant decrease (P<0.05) when compared to Group 3. There was a significant increase (P<0.05) in eosinophil count of Groups 4 and 5 when compared to Group 1 and a significant decrease (P<0.05) when compared to Group 3. A significant increase (P<0.05) was also observed in basophil count of Groups 4 and 5 when compared to Groups 1 and 2, alongside a significant decrease (P<0.05) when compared to Group 3. There was a significant decrease (P<0.05) in RBC (red blood cell) count of Groups 4 and 5 when compared to Groups 1

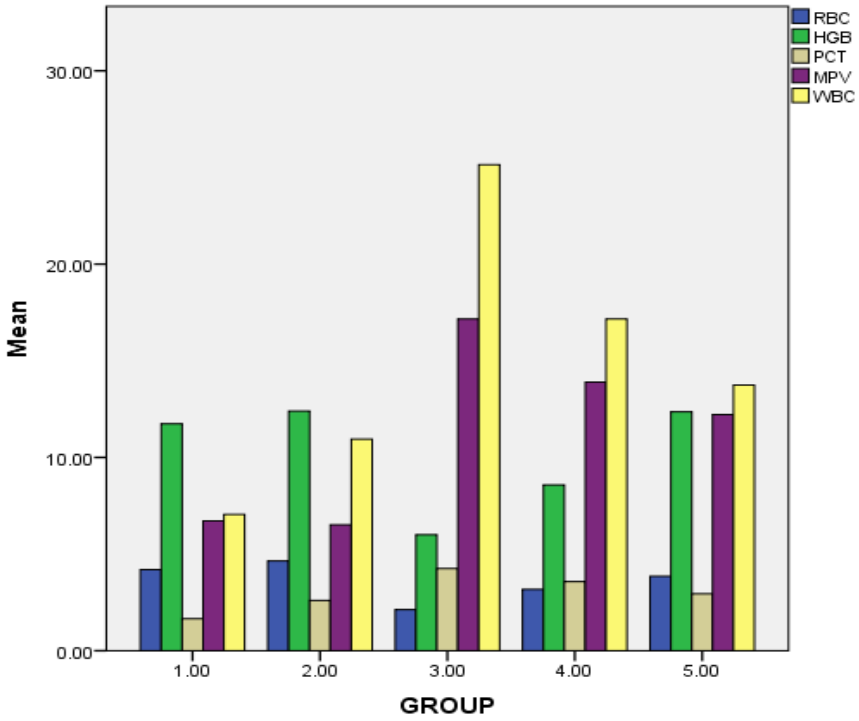

Figure 4. Graph of haematological indices (WBC, RBC, HGB, PCT, MPV).

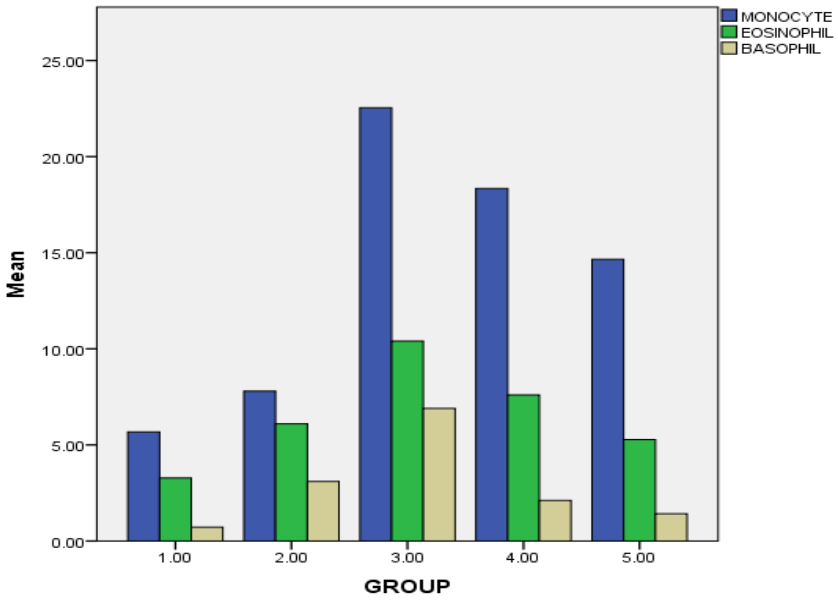

Figure 5. Graph of haematological indices (Monocytes, Eosinophils, Basophils).

and 2 shown in Table 6 and Figure 4. The haemoglobin (HGB) level was significantly decreased ($p<0.05$) in Groups 4 and 5. There was no significant ($P>0.05$) difference in haemoglobin count (HGB) concentration. There was a significant increase ($P<0.05$) in HCT (haematocrit) count of Groups 4 and 5 when compared to Group 3. There was a significant increase ($P<0.05$) in MCV (mean cell volume) and MCHC (mean corpuscular haemoglobin count) of Groups 4 and 5 when compared to Group 3 shown in Table 6 and Figure 6. There was a significant ($P<0.05$) increase in platelet count of Groups 4 and 5 when compared to Groups 1 and 2; the platelet

count was significantly decreased (P<0.05) when compared to

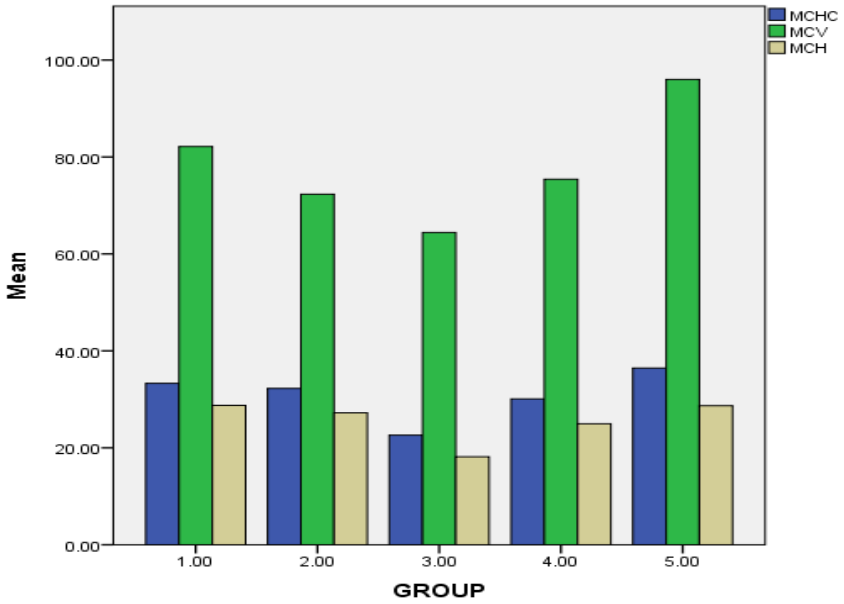

Figure 6. Graph of haematological indices (MCH, MCHC, MCV).

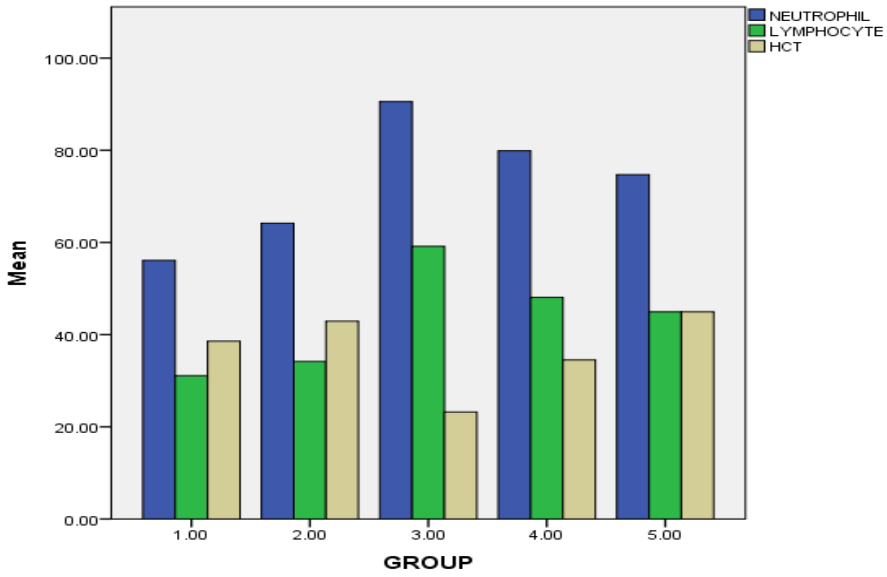

Figure 7. Graph of haematological indices (Neutrophils, Lymphocytes, Haematocrit).

Group 3. There was significant (P<0.05) decrease in mean platelet volume (MPV) count of Groups 4 and 5 when compared with Groups 1 and 2 and a significant increase when compared to Group 3.

DISCUSSION

Induction of diabetes using alloxan has been described as a useful experimental model for studying the effect of anti-diabetic agents (Szudelski, 2001). Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals result in the destruction of pancreatic β-cells and diabetes (Szudelski, 2001). Effect of treatment on body weight of control and test groups shows variation in the body weight of rats within the experimental period after

treatments; the results from this study revealed significant loss of weight in the untreated diabetic rats which is in accordance with the works of other researchers (Sharma

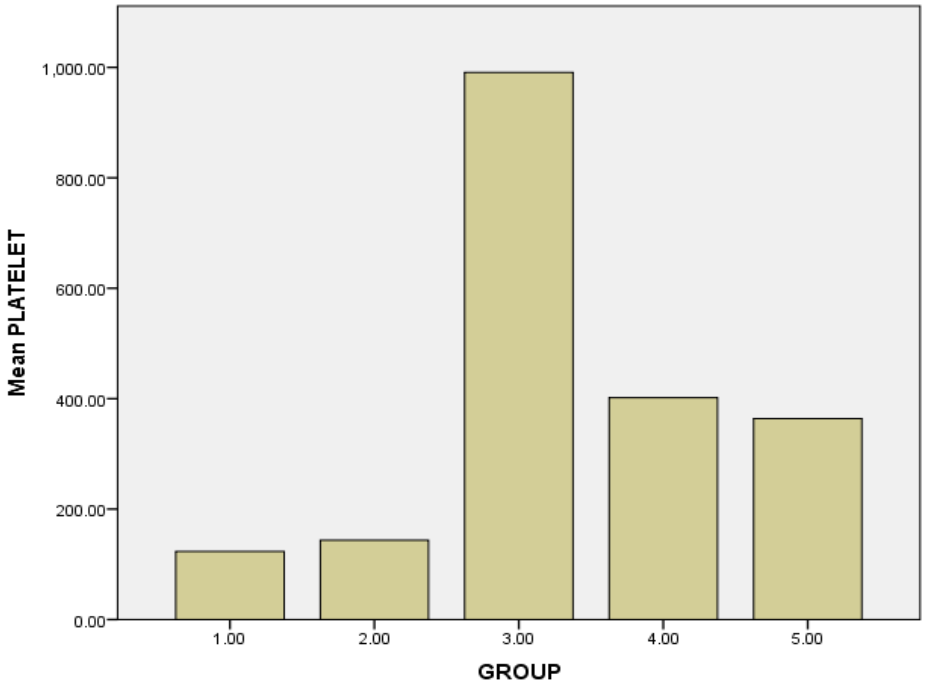

Figure 8. Graph of platelet count.

et al., 2010; Rao et al*.,* 2010). There were significant increases in the body weight of the treated diabetic animals (both the positive control and those treated with the n-Hexane fractions), which was comparable to that of the normal control (Nwachukwu et al*.,* 2010). This loss in weight of the untreated diabetic animals is attributed to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids (Granner, 2010). Rats that received the 400 mg/kg n-Hexane fraction showed a greater percentage of increase in body weight (22.01%) when compared to those that received the 300 mg/kg (12.55%) which is in line with the work of other researchers on different doses of the plant product (Iweala et al*.,* 2013).

Induction of diabetes using alloxan increased the blood glucose levels in the induced rats to about three to five times level in the normal control group. Destruction of the pancreatic beta cells by alloxan results in impaired insulin production which also results in increased blood glucose levels as insulin is responsible for the uptake of glucose from the bloodstream. The untreated diabetic group is characterized by an increase in blood glucose levels that is significantly different from that of the normal control. Reduction of fasting blood glucose levels of positive control and test groups reveals that oral administration of glibenclamide and the n-Hexane leaf fraction of *G. latifolium* had a hypoglycaemic effect on the diabetic rats. For the test groups, this hypoglycaemic effect was higher in the group administered 400 mg/kg of the n-Hexane fraction and less in the group administered 300 mg/kg of the extract; this is in accordance with the works of Abubakar et al. (2013). Some phytochemical constituents of the leaves of *G. latifolium* include tannins, flavonoids, saponins and alkaloids (Sharma et al*.*, 2010). These phytochemical constituents are known to have anti-hyperglycaemic effect (Sharma et al*.*, 2010) and so could be responsible for the anti-hyperglycaemic effect of *G. latifolium* leaves. Although the exact mechanism of action of the n-Hexane fraction of *G. latifolium* is unknown, the effect exhibited suggests a possible stimulation of insulin release from the residual β-cells and glucagon inhibition (Morebise et al*.,* 2002). In addition, the extract might have insulin-like effect by improving the glucose uptake and metabolism or by inhibiting gluconeogenesis thereby exerting the hypoglycaemic effect (Morebise et al*.*, 2002). However, this hypoglycaemic effect was not greater than that of the group that was administered the standard anti-diabetic drug (glibenclamide), this group showed a reduction in blood glucose levels to near normalcy when compared with the normal control. This is due to the insulin-stimulating actions of glibenclamide on the beta cells of the pancreas (Srinivasan et al*.,* 2012). It was obvious from this study that the standard diabetic drug (glibenclamide) proved more effective than the n-Hexane fractions of *G. latifolium* in controlling blood glucose levels in diabetes, probably because this a pure drug.

Assay for liver enzymes namely ALT, AST and ALP is important in assessing optimal liver function during

diabetes. Increase in the level of liver enzymes in the plasma is an indication of liver dysfunction (Dame, 2011). The increase in ALT and ALP levels in the groups that were administered with the n-Hexane fraction of *G. latifolium* fraction (both 300 and 400 mg/kg) were not significantly different from the normal control group, indicating possible hepato-protective effect of the plant fractions. There was no significant difference between the ALT and ALP levels of the positive control (glibenclamide) and that of the extract treated groups, which indicate the effectiveness of the fractions. There was a significant increase (in ALT and ALP levels) between the diabetic control (for ALP and ALT) when compared to the normal group. This means there was increased ALT and ALP activity in the serum, indicating possible hepatocellular injury or death. For AST, there were significant differences between normal and positive controls and the extract treated groups, implying that there was no significant reduction in the AST levels of the extract treated groups when compared with the normal control and positive control groups. AST levels difference between the treated groups and the negative control was not significant, which means the fractions were not so effective in reducing AST levels. AST is not necessarily an indicator of liver dysfunction because it is found in a variety of tissues including liver, heart, muscle, kidney and the brain and is released into the serum when any of these tissues are damaged. For example, AST level in serum could also be elevated in heart attacks or with muscle injury and is therefore not a highly specific indicator of liver injury as its elevation can occur as a result of other injured tissues (Davis and Shiel, 2017).

Pancreatic alpha amylase is a key enzyme in the digestive system that catalyzes the breakdown of complex carbohydrates into absorbable glucose. Inhibition of pancreatic α-amylase is one of the therapeutic approaches for the control of hyperglycaemia in diabetes (Etim et al*.,* 2008). The n-Hexane fraction of *G. latifolium* is seen to markedly inhibit the α-amylase levels in this study, which shows that *G. latifolium* may be useful in controlling postprandial hyperglycaemia in diabetic patients.

During diabetes, the levels of serum lipids (cholesterol, free fatty acids and phospholipids) are usually elevated. Induction of diabetes in this study caused significant increase in TC, TG, LDL, VLDL and a decrease in HDL compared to the normal control but the intervention with the n-Hexane fraction of *G. latifolium,* however reduced these indices (while increasing that of HDL); this was in consonance with the works of these researchers (Ugochukwu et al*.,* 2011, 2012*,* 2013). Diabetic state appears to be associated with increased synthesis of cholesterol. It has been hypothesized that hyperphagia of diabetes induces increased activity of HMG-CoA reductase of the intestine resulting in increased synthesis of cholesterol leading to raised levels in plasma (Christopher et al*.,* 2011). The hypertriglyceridemia may be due to i) higher rates of production of triglyceride rich VLDL by the liver (Nikkila and Kekki, 2013) and ii) decreased removal of TG by peripheral tissues - primarily adipose tissue and muscle. Insulin deficiency leads to high TG production and subsequent high packaging in VLDL. Lower HDL-C in diabetes may be due to reduced lipoprotein lipase activity (Nikkila and Kekki, 2013), an enzyme that breaks down fat in the form of triglyceride. LDL production rates are reportedly elevated in type 1 but return to normal after insulin infusion (Howard, 2007). It may be due to increased synthesis of VLDL or impaired removal of VLDL remnants. Impaired receptor mediated clearance of LDL has also been postulated (Howard, 2007). Treatment of the diabetic rats (Groups 4 and 5) with the n-Hexane fraction of *G. latifolium* reversed the hyperlipidaemic effect of diabetes but was not as effective as the standard drug. This indicates that the plant has hypolipidemic properties.

The result also showed that administration of the n-Hexane fraction of the plant seems to suppress the haematopoietic system. There was a significant increase in WBC and differential WBC count which was not readily reversed by the administration of the n-Hexane fraction of *G. latifolium*; this is in accordance with the work of Balogun et al. (2016). This increase in WBC count is because WBC, which is a marker of inflammation indicate a worsening of insulin action. The plant which is said to have insulin-stimulating action could only slightly reverse the elevated WBC count. RBC and HGB decreased significantly in the negative control. This is so because the hormone that regulates red blood cell production, erythropoietin (EPO) is produced by the kidneys and renal damage is one of the several complications of diabetes. If the kidney is affected, the production of this hormone will also be affected resulting in less production of RBC. Reduced RBC directly results in reduced haemoglobin levels. Groups 4 and 5 showed no significant increase despite the different doses administered. This might be due to the presence of saponin, which has been reported to reduce haematological parameters due to lysis of blood cells or suppression of blood cell synthesis (Irvine 2011; Schneider et al*.*, 2013). The mechanism that underlies the association between diabetes and haematocrit levels is unclear (Wannamethee et al*.,* 2004). One explanation for the decreased level of haematocrit in diabetes mellitus may be the effect of insulin-like growth factor-1 (IGF-1) on red blood cell development. IGF-1 which is associated with insulin resistance may inhibit haematopoiesis, thus decreasing haematocrit levels. The n-Hexane fraction of *G. latifolium* did not have much effect on the haematocrit levels and this agrees with the work of Balogun et al. (2016). For MCHC, this report is in agreement with a previous study (Ugochukwu et al*.,* 2013) which observed that MCHC values are lower in diabetic patients than in control groups indicating the presence of anaemia in the diabetic group as earlier established. However, in this

study, only the high dose of the extract significantly reduced MCHC, and is in accordance with the work of Balogun et al. (2016). Elevated platelet count is seen in the untreated diabetic rats and this has a positive correlation with the increased WBC count. Insulin antagonizes platelet reactivity, thus relative deficiency of insulin would be expected to increase platelet reactivity (David, 2010). Increased platelet and WBC count contributes to vascular events in diabetes. This is in agreement with previous reports (Ugochukwu et al., 2013). Increase in platelet count could be as a result of stress response and elevated platelet count could be injurious to the microcirculation and enhance the risk for vascular complications. The n-Hexane fraction of *G. latifolium* significantly decreased platelet count and this is suggested to be due to its insulin-stimulating effect on the pancreas (Srinivasan et al., 2012).

Conclusion

In light of this research, it could be stated that the n-Hexane fraction of *G. latifolium* has beneficial effects on blood glucose, lipid profile, liver and pancreatic enzymes hence improving metabolic aberrations. The results of the haematological indices also indicate that the presence of saponin suppresses hematopoiesis therefore incessant consumption of the plant is not advisable.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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