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THE EFFECT OF AQUEOUS LEAF FRACTION OF *Gongronema latifolium* ON ALLOXAN-INDUCED DIABETIC MODELS

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ABSTRACT

Gongronema latifolium is a medicinal plant used in indigenous system of medicine as an antidiabetic agent. Hypoglycaemic, hypolipidemic and haematological activities of aqueous leaf fraction of *Gongronema latifolium* in diabetic rat models were evaluated using standard analytical methods. Twenty-five animals were divided into 5 groups, all diabetic except group 1; Group I (normal control), group II (positive control), group III (negative control), while groups IV and V were administered 300mg/kg and 400mg/kg of the aqueous leaf fraction of the plant respectively. The result of the body weight of groups IV and V showed significant increase ($p < 0.05$) when compared to group III. The blood glucose level in group IV animals decreased significantly ($p < 0.05$) by 61.58% while group V further decreased by 66.11% when compared to group I (3.78%) and group III (19.79%). Diabetes induction caused significant increase ($p < 0.05$) in total cholesterol (TC)(3.80 ± 0.16mmol/l) and low-density lipoprotein cholesterol (LDL-C)(3.30 ± 0.19mmol/l)in group III when compared to normal control (TC=3.58 ± 0.02mmol/l, LDC-C=0.58 ± 0.04mmol/l), while treatment with aqueous fraction of *Gongronema latifolium* significantly decreased ($P < 0.05$) the level of TC(300mg/kg=3.74 ± 0.16mmol/l, 400mg/kg=3.54 ± 0.33mmol/l and LDL-C(300mg/kg=0.86 ± 0.10mmol/l, 400mg/kg=0.72 ± 0.04mmol/l)when compared to group III. The amino transferases (ALT and AST) parameters activities significantly increased ($P < 0.05$) in the diabetic group (AST=69.80 ± 4.83(µ/L), ALT=25.60 ± 3.70(µ/L)) but became reduced upon treatment with aqueous fraction of *Gongronema latifolium;* (ALT -300mg/kg=26.00 ± 1.30(µ/L), 400mg/kg=29.60 ± 4.04µ/L; AST -300mg/kg=60.60± 2.50µ/L, 400mg/kg=58.80 ± 1.59µ/L). Haematological indices such as WBC, RBC, HBG, HCT, PCV, also reveal significant changes with treatment of the aqueous leaf fraction. Thus, the result suggests that the aqueous leaf fraction of *Gongronema latifolium* could ameliorate hyperglycemic and hyperlipidemic activities and haematological disorders.

KEYWORDS: Gongronema Latifolium, Aqueous Fraction, Hypoglycaemic, Hypolipidemic and Haematological Indicies.

INTRODUCTION

Medicinal plants are those plants that are used in the treatment of diseases (Nwachukwu *et al.*, 2010). The tropical rainforest is the most biologically varying ecosystem on earth and it is enriched with enormous natural plant resources with rich dietary and medicinal properties utilized locally in folkloric medicine (Owolabi *et al.*, 2007). Although modern medicine may be available in developing countries, the use of herbs in treatment of diseases has often gained popularity for historical and cultural reasons (Nwangwu *et al.*, 2009), making traditional medicine an unavoidable global acceptance.

Gongronema latifolium is a tropical rainforest plant which belongs to the family Asclepiadaceae and genus Gongronema. (Osuagwu *et a*l., 2013). It is commonly grown in West Africa and is locally called "Utasi" by the Ibibios, "Utazi" by the Igbos in South East and "Arokeke" by the Yorubas in South Western part of

Nigeria (Ugochukwu and Babady, 2002; Ugochukwu *et al*., 2003). It is an edible plant with green leaf, yellow flower and stem that produces milky latex when cut. It has a characteristic sharp, bitter and slightly sweet taste; it is mainly used as vegetable, medicine or spice by the people (Nwachukwu *et al*., 2010).

Diabetes mellitus is a disease where there is too much sugar (glucose) floating in the blood. This occurs because either the pancreas cannot produce enough insulin or the cells in the body have become resistant to insulin. It is a symptom of high blood sugar which includes frequent urination, increased thirst, and increased hunger (Balogun *et al*., 2016). Diabetes tends to damage the cell membranes which results in an elevated production of reactive oxygen species (ROS). The generation of ROS appears to play a critical role in the pathogenesis of diabetes mellitus (Harnett *et al*., 2000). Diabetes if left untreated can cause many complications which could include: diabetic ketoacidosis, non ketotic hyperosmolar coma, or death. Serious long-term complications include: heart disease, stroke, chronic kidney failure, and damage to the eyes.

One of the most potent methods to induce experimental diabetes mellitus is the chemical induction by Alloxan. It is a well-known diabetogenic agent that is used to induce type 1 diabetes in experimental animals (Etuk, 2010). Alloxan (2,4,5,6-tetra-oxypyrimidine; 2,4,5,6-pyrimidinetetrone) 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, resulting in consequent lack of insulin secretion. This causes an insulin-dependent diabetes mellitus with characteristics similar to type 1diabetes in humans.

Lipid profile can be described as a direct measure of three blood components which include: cholesterol, triacyglycerol and high- density lipoproteins. Cholesterol is a vital substance that the body uses to produce such things as digestion- aiding materials, hormones and cell membranes. The cholesterol and triacyglycerol are transported in the body by a combination of lipid and proteins to form lipo-proteins. The high-density lipoprotein is lipo-proteins made mostly of the protein and cholesterol. This type of cholesterol aids in the clearing of cholesterol deposits in the blood vessels which were left by another blood component known as the low- density lipoprotein (LDL). LDLs level may be calculated from three directly measured lipids or may be more accurately measured by direct test (Dinsmoor, 2013).

The liver plays an important role in the maintenance of normal blood glucose levels during fasting as well as in the postprandial period, and its role in the pathogenesis of diabetes especially type 2 diabetes mellitus has attracted much interest (Marchesini *et al*., 2001). Alanine transaminase (ALT) and aspartate transaminase (AST) are transaminase enzymes found in the liver, they are also called aminotransferases. Increased activities of these liver enzymes are indicators of hepatocellular injury. They are among the most sensitive and widely used liver enzymes (Davis and Shiel, 2017). Plant products and its evaluation is a growing interest as these plants are known to contain many bioactive substances with therapeutic potentials. There is need therefore to investigate on the effects of aqueous fraction of *Gongronema latifolium* leaf"s efficacy as an antidiabetic.

MATERIALS AND METHODS

Chemical Reagents: Lipid profile- triacyglycerol (TG), Total cholesterol (TC), High density lipoprotein (HDL), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were obtained from the chemical methods using kits from Randox Laboratories Ltd, Admore Diamond Road, Crumlin, Co. Antrim, United Kingdom, Qt 94QY, Low density lipoprotein cholesterol (LDL-C) and Very low -density lipoprotein cholesterol (VLDL-C) were obtained through calculations from the other parameters (Tietz, 2008). Alloxan monohydrate was obtained from sigma Aldrich, Switzerland, while glibenclamide was obtained from Swiss pharm. Nig. Ltd.

Collection and Identification f Plant Materials: Fresh but matured *Gongronema 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 coloured bottle and kept for 72 hours under 4^{O}C in a dark compartment. On the third day, the solution was filtered first with a cheese cloth and then with Whatman No. 1 filters paper. Filtrate gotten was concentrated in vacuo with a rotary evaporator at 37-40^{O}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. The crude extract was dissolved with 20ml distilled water in a beaker and the different fractions were gotten following polarization gradient. The sediment (mother liquor) was dissolved in 400ml of distilled water and the mixture was poured into a separating funnel (1000ml) and shaken vigorously. It was allowed to stand for about 4 hours for maximum extraction and then filtered. The supernatant was evaporated to dryness using a water bath leaving behind the aqueous fraction.

Experimental Animals: Twenty-five male Albino Wistar rats were used in the experimental analysis. The animals were obtained from the Basic Medical Sciences animal house of the University of Uyo, Nigeria and were housed in clean cages (wooden bottom and wire mesh top) at the Department of Pharmacology and Toxicology, Faculty of Pharmacy of the University of Uyo, Nigeria. The animals were maintained under standard laboratory condition of humidity (50±5%) and temperature (28±2ºC), and a 12hours light/dark cycle. The animals had free access to feeds and water all through the experimental period and were acclimatized for 14days before the commencement of the experimental research. The handling of the animals was approved by the Animal Ethical Committee of the University of Uyo, Uyo, Nigeria.

Induction of Experimental Animals with Diabetes: Diabetes was induced by single intraperitorieal injection of freshly prepared solution of alloxan monohydrate (150mg/kg bodyweight) 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 hours. The 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 200mg/dl were selected for the study.

Experimental Design: The experimental design consists of 25 Albino Wistar rats which were divided into five different groups, each of which contained 5 rats. The animals in groups I, II, and III were designated Normal, Positive and Negative Control and were given 2ml distilled water, 5mg/kg glibenclamide and 2ml distilled water respectively, while the Plant test groups 4 and 5 were gavage graded doses of 300 and 400mg/kg of the aqueous leaf fraction of *G. latifolium*. The blood glucose level was monitored in the diabetic rats by tail tipping method using fine test glucometer (glucose dye oxidoreductase mediator reaction) method. 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 shown in the table below.

Table. 1: Experimental Design.

Groups	No of animal	Conditions	Treatments	Dose
Normal control (I)	5 rats	Non- Diabetic	Distilled water	2ml
Positive control (II)	5 rats	Diabetic	Glibenclamide	5mg/kg
Negative control (III)	5 rats	Diabetic	Distilled water	2ml
Fraction of *G.l* (IV)	5 rats	Diabetic	Aqueous fraction of G.l	300mg/kg
Fraction of *G.l* (V)	5 rats	Diabetic	Aqueous fraction of G.l	400mg/kg

G.l: Gongronema latifolium.

Collection of Blood Sample for Experimental Analysis: At the end of the 14 days, feeds were withdrawn from the animals and they were fasted overnight but with free access to water. They were then euthanized under chloroform vapour and sacrificed. Immediately whole blood was collected for sera preparation via cardiac puncture using sterile syringes and needles, emptied into plain tubes and allowed to clot for about two hours. Centrifugation of clotted blood was done using bench top centrifuge (MSE Minor, England), serum was separated with sterile syringes and stored frozen until needed for analysis.

Biochemical Assays: The method involves using the kits and a chemical auto analyser (Model: Mindray BS-120/130) (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Vienna).The biochemical parameters assayed were; blood glucose levels, liver and pancreatic enzymes, lipid profiles and haematological indices.

Statistical Analysis: The results were expressed as mean ±SD and test of statistical significance were carried out using one-way ANOVA. The differences between mean values were analyzed by Microsoft Excel Xl toolbox (2.6version) at $P < 0.05$ level of significance.

RESULTS

Effects of Aqueous Leaf Fraction of *Gongronema latifolium* on Body Weight of Diabetic and Non-Diabetic Rats: Results obtained in table 2 showed that the body weight of group III rats significantly decrease ($P<0.05$) when compared to the group I. The effect of administration of aqueous fraction of *Gongronema latifolium* on body weight of groups 4 rats shows a significant increase ($P<0.05$) when compared to the group III; and a further increase in weight was observed in group V when compared to groups I, II and III.

Table. 2: Effect of Aqueous Leaf Fraction of *Gongronema latifolium* on Body Weight of Diabetic and Non-Diabetic Rats.

Group	Initial body weight	Final body weight	Weight change %
Group I(normal control)	124.60±3.80	129.80±6.14	4.17
Group II(positive control)	125.20±3.81	137.45±5.34	9.78
Group III(negative control)	121.60± 5.08	105.54±4.05	-13.20
Group IV 300mg/kg	128.54±6.23	139.80±7.35 [a, c]	8.75
Group V 400mg/kg	130.31±3.20	143.60±3.20 [a, b c]	10.19

Results are presented as Mean ± SEM, n = 5
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

Effects of Aqueous Leaf Fraction of *Gongronema latifolium* on Blood Glucose Level of Diabetic and Non-Diabetic Rats: The effect of blood glucose level as shown in table 3 shows the effect of aqueous leaf fraction of the *Gongronema latifolium* plant. In group II, a significant decrease ($P \leq 0.05$) in the blood glucose level was observed when compared to group III, while group IV shows a significant decrease ($P \leq 0.05$) in the blood glucose level when compared to groups I and III. Group V shows a further significant decrease ($P \leq 0.05$) in the blood glucose level as shown in Table 3.

Table. 3: Effect of Administration of Aqueous Leaf Fraction of *Gongronema latifolium* on Blood Glucose Concentration of Diabetic and Non-Diabetic Rats.

Group	Initial Blood Glucose Level (mg/dl)	Final Blood Glucose Level (mg/dl)	Blood Glucose Level Percentage Change (%)
Group I (Normal)	81.40 ± 2.30	78.32 ± 1.18	3.78
Group II (Positive control)	399.45 ± 0.09	132.76 ± 10.81	66.67
Group III (Negative control)	303.61 ± 1.72	273.53 ± 15.64	19.79
Group IV (300mg/kg)	348.98 ± 1.25	134.08 ± 13.55[a, c]	61.58
Group V (400mg/kg)	371.06 ± 0.54	125.74 ± 5.08[a, c]	66.11

Results are presented as Mean ± SEM, n = 5
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

Effects of Aqueous Leaf Fraction of *Gongronema latifolium* on Liver and Pancreas Enzymes of Diabetic and Non-Diabetic Rats: The aqueous fraction of *Gongronema latifolium* treated group showed significant changes ($P < 0.05$) in serum enzymes concentration when compared to group III; alanine amino transaminase enzyme (ALT) significant increase ($P < 0.05$) in group II in comparison to group I. Treatment with 300mg/kg of the aqueous fraction plant showed a significant increase ($P < 0.05$) in the ALT enzyme, at 400mg/kg, a further increase in the enzyme was observed, when compared to groups I and III as shown in the table 4. Alanine phosphatase enzyme (ALP) showed significant increase ($P < 0.05$) with the *Gongronema latifolium* treated diabetic rats, when compared to group III. Pancreas α-amylase showed significant decrease ($P < 0.05$) in groups IV and V when compared to groups II and III. This change was also observed when compared to group I.

Table. 4: Result of Administration of Aqueous Leaf Fraction of *Gongronema latifolium* on Liver Enzymes and Alpha Amylase of Diabetic and Non-Diabetic Rats.

Group	ALT(µ/L)	ALP(µ/L)	AST(µ/L)	Alpha Amylase(µ/L)
Group I (Normal control)	19.40 ± 1.29	72.00 ± 2.74	66.80 ± 7.55	359.80 ± 31.08
Group II (Positive control)	31.80 ± 2.35	76.80 ± 1.71	60.80 ± 2.75	258.20 ± 21.15
Group III (Negative control)	25.60 ± 3.70	68.20 ± 0.73	69.80 ± 4.83	292.80 ± 17.84
Group IV (300mg/kg)	26.00 ± 1.30[b]	70.60 ± 1.72	60.60± 2.50[a,b,c]	333.40 ± 14.43
Group V (400mg/kg)	29.60 ± 4.04[a]	70.00 ± 2.00	58.80 ± 1.59	333.20 ± 19.23

Results are presented as Mean ± SEM, n = 5
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

Effects of Aqueous Leaf Fraction of *Gongronema latifolium* on Lipid Profile of a Diabetic and Non-Diabetic Rats: Total cholesterol (TC) concentration shows a significant increase ($P<0.05$) in group III when compared to group I, on treatment with aqueous fraction of *Gongronema latifolium*, significant decrease ($P<0.05$) was observed when compared to group III. High density lipoprotein (HDL) in groups IV and V showed significant increase ($P<0.05$) in concentration when compared to group III. Low density Lipoprotein (LDL) concentration shows the opposite of the HDL. There was significant decrease ($P<0.05$) in the *G. latifolium* treated diabetic rats when compared to group III. Triglyceride (TG), and Very low-density lipoprotein (VLDL) of groups IV and V shows significant increase($P<0.05$) in concentration when compared to groups I and III as seen in Table 5.

Table. 5: Effect of Aqueous Leaf Fraction of *Gongronema latifolium* on Lipid Profile of Diabetic and Non-Diabetic Rats.

Group	Total Cholesterol (mmol/l)	Triglyceride (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)
Group I (Normal)	3.58 ± 0.02	1.44 ± 0.09	2.46 ± 0.22	0.58 ± 0.04	0.46 ± 0.10
Group II (Positive control)	3.24 ± 0.09	1.70 ± 0.07	1.66 ± 0.10	0.72 ± 0.09	0.72 ± 0.08
Group III (Negative control)	3.80 ± 0.16	1.74 ± 0.09	0.70 ± 0.07	3.30 ± 0.19	0.76 ± 0.05
Group IV (300mg/kg)	3.74 ± 0.16[b]	1.98 ± 0.19[a]	1.94 ± 0.30[c]	0.86 ± 0.10[c]	0.85 ± 0.08[a]
Group V (400mg/kg)	3.54 ± 0.33	1.38 ± 0.12	2.14 ± 0.38[c]	0.72 ± 0.04[c]	0.62 ± 0.05

Results are presented as Mean ± SEM, n = 5
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

Effects of Aqueous Leaf Fraction Fraction of *Gongronema latifolium* on Haematologic Indices of Diabetic and Non-Diabetic Rats: Red blood cells (RBC) increased in concentration in group III when compared to group I. On treatment with aqueous fraction of *Gongronema latifolium* (RBC) count significantly decreased ($P< 0.05$) when compared to groups I and III. Also significant decreases ($P< 0.05$) in the levels of white blood cell (WBC) count, platelet count, packed cell volume (PCV) and haemoglobin (Hb) concentration were observed in groups IV and V, when compared to group III as shown in table 6. The mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly increased ($P< 0.05$) when compared to group III, while the mean corpuscular haemoglobin concentration (MCHC) was only significantly increased ($P< 0.05$) in groups IV and V when compared to group III as shown on Table 6.

Table. 6: Effect of Aqueous Leaf Fraction of *Gongronema latifolium* on Hematological Indices of Diabetic and Non-Diabetic Rats.

Group	Group I (Normal)	Group II (Positive control)	Group III (Negative control)	Group IV (300mg/kg)	Group V (400mg/kg)
WBC(x10^9/L)	13.05 ± 0.61	13.95 ± 0.68	11.14 ± 0.39	13.59 ± 0.87[c]	13.86± 0.56[a,b,c]
Neutrophils (%)	24.12 ±1.35	22.18 ±1.72	33.56 ± 5.22	24.72 ± 1.24[c]	22.76 ± 3.26[c]
Lymphocyte (%)	71.10 ±1.17	72.20 ±1.72	59.16 ± 5.19	71.08 ± 1.35[c]	68.92 ± 3.41[c]
Monocyte (%)	2.68 ± 0.15	2.80 ± 0.13	3.54 ± 0.30	2.16 ± 0.49[c]	2.52 ± 0.68
Eosinophils(%)	1.28 ± 0.04	1.10 ± 0.25	1.40 ± 0.14	1.00 ± 0.25	1.20 ± 0.15
Basophils (%)	0.72± 0.06	1.0±0.07	0.90 ± 0.32	0.68 ± 0.12	0.88 ± 0.14
RBC(x10^12/L)	6.20 ± 0.18	6.64 ± 0.27	7.32 ± 0.30	6.70 ± 0.27	6.42 ± 0.13[c]
HGB(g/dl)	11.74 ± 0.26	12.40 ± 0.35	13.00 ± 0.58	11.98 ± 0.39	12.40 ± 0.20
HCT (%)	38.60 ±0.92	42.92 ±0.86	46.20 ± 2.15	42.78 ±1.03[a,c]	41.68 ± 0.61[c]
MCV(fl)	62.14 ± 0.94	62.32 ± 1.19	62.36 ± 1.13	63.18 ± 1.61	64.42 ± 1.19
MCH(pg)	18.76 ± 0.24	17.24 ± 1.39	18.16 ± 0.27	18.44 ± 0.33	18.60 ± 1.42
MCHC(g/dl)	30.30 ± 0.23	29.26 ± 0.31	28.60 ± 0.35	29.28 ± 0.32	29.26 ± 0.38
Platelet(x10^9/L)	713.20±74.21	653.60±46.56	616.80± 26.23	714.80± 53.64	614.80± 29.92
MPV(fl)	6.72 ± 0.19	6.52 ± 0.21	7.16 ± 0.09	6.06 ± 0.12[c]	6.36 ± 0.59[c]
PCT	4.66 ± 0.40	4.16 ± 0.08	4.25 ± 0.24	4.32 ± 0.39	3.89 ± 0.19

Results are presented as Mean ± SEM, n = 5, 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.

DISCUSSIONS

The plant kingdom has become a very useful resource for man in his desire and search for beneficial products for nutritional and or medicinal purposes (Udoh, *et al.*, 2013). These effects are present because they contain some bioactive compounds (Effiong, *et al.*, 2012).

Diabetes mellitus is a metabolic disorder affecting about 5-10% of the world's population (Xie *et al*., 2011; Patel, *et al*., 2012). It is a disease condition characterized by alterations in carbohydrate, lipid and protein metabolism (Das *et al.*, 1996). More than 400 plant species have demonstrated hypoglycaemic activity (Verspohl, 2002; De Souse *et al*., 2004; Colca, 2006; Patel *et al*., 2012). This makes further research efforts a pressing need to discover new antidiabetic agents from natural plants. *Gongronema latifolium* is a typical example of such antidiabetic plant. According to Pouwer and Hermanns (2009) the management of diabetes should focus on three main targets: prevention of hyperglycaemia and its associated complications, prevention of hypoglycaemia and maintenance of the patient's quality of life. This paper research work focus on the antidiabetic activity of aqueous leaf fraction of *Gongronema latifolium* on alloxan induced diabetic models. Significant changes in weight, blood glucose level, lipid profile and haematological indices are the parameters assessed on this research work.

Weight changes of diabetic rats in group 1 compared to group III as observed in table 2.0 according to Granner, (1996) might be attributed to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids. Consumption of *G. latifolium* is readily associated with increase in weight (Iweala and Obidoa, 2009). As seen in Table 2, at doses of 300mg/kg and 400mg/kg, consumption of the aqueous fraction of *G latifolium* results in a significant increase ($P<0.05$) in body weight of the experimental animals.

Administration of aqueous leaf fraction of *Gongronema latifolium* to the experimental animals reveals significant decrease ($P<0.05$) in the blood glucose level when compared to group III; negative control, hence showing hypoglycaemic effect. Group II showed a significant decrease ($P<0.05$) in blood glucose level due to its insulin- stimulating actions on the beta cells of the pancreas (Srinivasan *et al*., 2008), when compared to group I and III. Oral administration of the aqueous leaf fraction reveals steady significant decrease ($P<0.05$) in group IV and a further significant decrease ($P<0.05$) in blood glucose level of group V when compared to group III.

Gongronema latifolium is known to contain various phytochemical constituents; indicating possible synergism between its components, the aqueous fraction was reported to have a higher antihyperglycaemic effect as seen in the activity in vivo in comparable insulinotropic effect to the anti-diabetic drug (glibenclamide) (Adebajo *et al*., 2012). Literature seems to indicate that *G. latifolium* acts to stimulate the secretion of insulin from the pancreas via non-phenolic molecules, the active principles responsible for *G. latifolium* a antidiabetic effect are yet to be fully characterized.

Assay for liver and pancreas enzymes; alanine amino transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and alpha (α) amylase is important in assessing optimal liver function during diabetes. Significant Increase ($P<0.05$) in the level of liver enzymes in the plasma is an indication of liver dysfunction (Dame, 1981). The increase in ALT and ALP showed statistical significant difference ($P<0.05$) from the control group (III), as shown in Table 4, indicating possible hepato-protective effect of the *G latifolium* plant. The AST level showed significant reduction ($P<0.05$) in group IV and V, when compared to group I. AST according to Iweala *et al.*, (2013) is not a good indicator of liver dysfunction and this further substantiates the possible hepato - protective effects of *Gongronema latifolium* in diabetes.

Pancreatic α-amylase is a key enzyme in the digestive system that catalyzes the initial step in hydrolysis of starch to maltose and finally to absorbable glucose. Degradation of dietary starch leads to elevated postprandial hyperglycaemia. Retardation of starch hydrolysis by inhibition of pancreatic α-amylase is one of therapeutic approaches for the control of postprandial hyperglycaemia in pre-diabetes, diabetes and obesity (Tundis *et al.*, (2010). *G. latifolum* is seen to markedly inhibit the action of pancreatic α-amylase in this study which is in accordance with the reports by Kwon *et al*., (2007) and Akkarachiyasit *et al*., (2011).

Lipid profile can be described as a direct measure of three blood components which include: cholesterol, triacylglycerol and high-density lipoproteins. Cholesterol is a vital substance that the body uses to produce such things as digestion- aiding materials, hormones and cell membranes. The high- density lipoprotein (HDL) in this research work showed significant increased ($P<0.05$) concentration in groups IV and V when compared to groups I and III, as seen in Table 5. The level of HDL in serum is inversely related to the incidence of myocardial infarction. As it is antiatherogenic or protective in nature, hence HDL is known to be "good cholesterol" (Vaudevan *et al.*, 2011). The level of low density lipoprotein (LDL) function in the transportation of cholesterol to the peripheral tissues, they are mostly derived from very low- density lipoprotein (VLDL) (Vaudevan *et al.*, 2011). LDL (bad cholesterol) is known to be deposited in tissues, and it has lethally dangerous lipoprotein (Vaudevan *et al.*, 2011). This research work as seen in fig 4.4 showed a significant decrease ($P<0.05$) in the LDL concentration with the *Gongronema latifolium* treated diabetic rats 300mg/kg and a further

significant decrease (P<0.05) at 400mg/kg when compared to the negative control group.

Red blood cell (RBC) counts significantly decreased (P<0.05) in groups IV and V when compared with group III. Also, significant decreases (P<0.05) in the levels of white blood cell (WBC) count, platelet count, packed cell volume (PCV) and hemoglobin (Hb) concentration were observed as in line with a research work by Edet *et al*., (2011), the decreases were dose dependent. The aqueous fraction showed significant effect on RBC count, PCV, MCV and MCH. Changes in haematological indices in experimental animals exposed to different chemical agents or extract-based active principles, since the same or closely related pattern of haematological indices was observed for Hb, PCV, and RBC in both diabetic and non-diabetic group. Within the diabetic group, significant decrease in Hb (P<0.05) level following increasing intragastric treatment with *G. latifolium*. This observation may be an attempt by the aqueous fraction to stop haemolysis of RBC caused by alloxan. Also, the significant increase (P<0.05) in WBC count in groups IV and V when compared to group III may be due to alloxan poisoning. This is in line with normal physiological response following the perception of an assault by the body defense mechanism (Edet *et al*., 2011).

CONCLUSION

The findings of this research work suggest clearly that *Gongronema latifolium* may possess hypoglycaemic effect thereby ameliorating the effects of hyperglycaemia and hyperlipideamia in diabetes mellitus. The studies also suggested the ability of aqueous leaf fraction of *Gongronema latifolium* to reduce activity of serum liver and pancreas enzymes and enhance antioxidant defiance status, as well as significant positive effect on haematological indices.

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