Available online on 15.09.2022 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Hepato and Nephro-protective Potentials of *Gongronema latifolium* in Streptozotocin Induced diabetic Rat Model

Gabriel Otu Ujong[1]*, Justin Atiang Beshel[2], Nkanu Etah Etah[1], Idara Asuquo Okon[3], Ofem Effiong Ofem[2]

[1] *Department of Human Physiology, Cross River University of Technology, Okuku Campus, Cross River State, Nigeria*

[2] *Department of Human Physiology, University of Calabar, Cross River State, Nigeria.*

[3] *Department of Physiology, PAMO University of Medical Sciences Port Harcourt, Rivers State, Nigeria*

Article Info:

Article History:

Received 18 July 2022
Reviewed 23 August 2022
Accepted 29 August 2022
Published 15 Sep 2022

Cite this article as:

Ujong GO, Beshel JA, Etah NE, Okon IA, Ofem OE, Hepato and Nephro-protective Potentials of *Gongronema latifolium* in Streptozotocin Induced diabetic Rat Model, Journal of Drug Delivery and Therapeutics. 2022; 12(5):70-79

DOI: http://dx.doi.org/10.22270/jddt.v12i5.5623

*Address for Correspondence:

Gabriel Otu Ujong, Department of Human Physiology, Cross River University of Technology, Okuku Campus, Cross River State, Nigeria

Abstract

Gongronema latifolium (GL) is reported to have hepato-protective and nephron-protective ability. Diabetes is associated with liver and kidney damage. This work was designed to evaluate the effect of ethanolic leaf extract of *Gongronema latifolium* on liver and kidney functions in Streptozotocin-induced diabetic rats. 40 Wistar rats of both sexes (150g-200g) were divided into five (n=8). Group 1- normal control (received normal saline placebo orally). Group 2 received 200mg/kg GL orally. Group 3 was the diabetic group administered 65mg/kg of streptozotocin (STZ) intraperitoneally for two consecutive days with an interval of one day in between. Group 4 (diabetic + GL group) had STZ (65mg/kg i.p.) + oral administration of 200mg/kg GL. Group 5 was the diabetic group treated with insulin subcutaneously. Blood samples were collected via cardiac puncture to assess Liver enzymes activity, Serum electrolytes biomarkers, Urea and Creatinine. The Result shows a significant ($P<0.05$) increase in the glucose level of the diabetic group compared to the control. Intervention with GL and insulin significantly reduced the glucose level towards normal. The liver enzymes activity was significantly ($p<0.05$) increased in the diabetic group but was significantly ($p<0.01$) decreased following treatment with GL and insulin. Sodium, potassium, chloride, urea and creatinine were significantly ($p<0.01$) increased in the diabetic group with a decrease in bicarbonate. GL and insulin significantly ($p<0.01$) reversed these changes in electrolytes and liver enzymes activity towards normal. Results presented shows that GL just like insulin possess hypoglycemic, nephro and hepato-protective potential. Thus, in diabetic condition, *Gongronema latifolium* leaf extract may exert ameliorative effect on these target organs.

Keywords: *Gongronema Latifolium,* Diabetes, insulin, serum electrolytes, liver enzymes, rats.

INTRODUCTION

Diabetes mellitus (DM) is a pathological and metabolic condition characterized by impaired glucose metabolism caused by inadequate insulin action or insulin resistance[1,2]. Clinically, it is defined as a fasting plasma glucose level >7.8 mmol/l (140mg/dl) or a 2hour post-prandial plasma glucose >11 mmol/l (200 mg/dl)[3]. In DM, blood glucose level is persistently raised above normal range (80-100mg/dl). It is classified into two; Type 1 and Type 2 diabetes with Type 2 being 10 times more common than Type-1[4]. It is a complicated and chronic disease with complex etiologies[5,6] which can lead to several pathological conditions such as impaired glucose tolerance, nephropathy, neuropathy, blurred vision, atherosclerosis, myocardial infarction, hypertension and stroke due to oxidative stress[7,8].

There is increased prevalence of DM due to population growth, aging, urbanization and lifestyle. Although lifestyle modification plays a greater role in the prevention of diabetes, effective clinical management of diabetes relies on adequate control of blood glucose, which must take into consideration the need to maintain adequate energy in the face of intermittent food intake along with variable exercise and thus variable demand[9]. It is a chronic lifelong condition that affects the body's ability to use the energy found in food. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030[10].

Gongronema latifolium (Asclepiadaceae) is a herbaceous climber with yellow flowers and stem that yields characteristic milky exudates. It is widespread in Tropical Africa and can be found from Senegal, Chad and Democratic Republic of Congo. It occurs in rainforest, deciduous, and secondary forest, and also in mangrove and disturbed roadside forests, from sea level up to 900m altitude[11,12]. The leafy vegetable can be propagated by seed. Its common name is 'amaranth globe'. In Nigeria, *G. latifolium* is known by different local names such as 'utasi' by the Efiks/Ibibios, '*utazi*' by the Igbos and *arokeke* by the Yorubas[13,14]. The phytochemical screening of GL reveals that the plant contains flavonoid, polyphenol, saponin, tannin alkaloid and mineral. It possesses antioxidant activity by increasing superoxide dismutase and glutathione peroxidase activities[15] and also reduces renal and hepatic oxidative stress, lipid peroxidation, and increases the glutathione/glutathione disulphide (GSH/GSSG) ratio[13,15].

G. latifolium crude leaf extract is used in the treatment of malaria, diabetes, hypertension, and as laxative[16].

Electrolyte imbalance is markedly present in patience with uncontrolled blood sugars level therefore, serum electrolyte should be routinely measured in patients with diabetes mellitus. Electrolytes play an important role in several body mechanisms. It helps maintain acid base balance, membrane potential, muscle contraction, nerve conduction and control body fluid. Alterations in electrolytes homeostasis may lead to physiologic disorders. Insulin has been shown to activate Na /K -ATPase enzyme. Therefore, low serum insulin level reduces Na /K -ATPase activity with poor Na and K metabolism as a result and so transport across bio-membranes as well as hindered monosaccharide uptake by intestinal epithelia occurs. In diabetes mellitus, hyperglycemia causes glucose induced osmotic diuresis with resultant loss of body fluids and electrolytes[17]. Several studies have estimated the electrolytes levels in diabetes mellitus in several countries and showed the association between electrolytes and hyperglycemia[18,19].

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time[20]. Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGT), and bilirubin act as markers of biliary function and cholestasis. Albumin and prothrombin reflect liver synthetic function.

Increased activities of liver enzymes such as (AST), alanine aminotransferase (ALT) alkaline transferase (ALP) are indicators of hepatocellular injury. Increased activity of these markers is associated to type 2 diabetes mellitus with a higher incidence of liver function test abnormalities than individuals who do not have diabetes[21]. Mild chronic elevations of transaminases often reflect underlying insulin resistance. Anti-diabetic agents have generally been shown to decrease alanine aminotransferase levels as tighter blood glucose levels are achieved. The aminotransferases AST and ALT are normally < 30-40 units/l. Elevations of aminotransferases greater than eight times the upper limit of normal reflect either acute viral hepatitis, ischemic hepatitis, or drug- or toxin-induced liver injury.

Although, studies have revealed the anti-diabetic properties of *Gongronema latifolium* there is paucity of information regarding its role in the liver and kidney of diabetics. Thus, this study was aim at investigating the hepatoprotective and nephroprotective effect of *Gongronema latifolium* leaf extract in Streptozotocin Induced diabetic rat.

The aim of this study was therefore to investigate the effect of *Gongronema latifolium* leaf extract on some liver and kidney functions in Streptozotocin diabetic rats

MATERIAL AND METHODS

Preparation of *Gongronema latifolium* extract

The preparation of extract was according to standard method[13]. *Gongronema latifolium* was harvested in a local farm in Ugep, yakurr Local Government, Cross River State. It was identified and authenticated in the Department of Botany and Ecological Studies, University of Calabar, Calabar. The leaves were washed and dried under shade, for seven days then blended into fine powder and stored in a cool dry place away from light until required for use. The powdered leaves (400g) was dissolved in 1250ml of ethanol (BDH Ltd Poole, England) in the evening, and allowed to stay overnight. The mixture was then centrifuged in the morning of the next day and the supernatant collected. The supernatant was suction filtered, first, using Whatmann no. 1 filter paper, and then a second time using cellulose filter paper. The filtrate was evaporated to dryness at 30°C using a vacuum rotatory evaporator (Caframo, VV2000, Ohio) and water bath (Caframo, WB2000). This extraction gave a percentage yield of about 4.3% using a digital sensitive weighing balance. The extract was stored at 4°C till further use.

Experiment animal design

Before the commencement of this study, ethical approval was obtained from the Faculty of Basic Medical Sciences University of Calabar Animal Research and Ethical Committee with ethical number No: 019PY20317. Forty adult Wistar rats of both sexes weighing 150-200 g were divided into 5 groups (n = 8). Group 1: Control, Group 2: received 65 mg/kg body weight of STZ (DM), Group 3: received 200 mg/kg body weight of *Gongronema latifolium* orally (GL) Group 4: received 65mg/kg body of STZ intraperitoneally followed by oral administration of *Gongronema latifolium* leaf extract orally (DM + GL), Group 5: received 65mg/kg body of STZ followed by intraperitoneal injection of insulin (DM + Insulin)

Administration of *Gongronema latifolium* extract and insulin

The extracts was administered according to standard method[15]. The plant extracts reconstituted in distilled water (vehicle) were administered via oral gastric intubation at a dose of 200 mg/kg body weight daily to groups 3 and 4 animals. Insulin (10 IU/kg body weight) was administered subcutaneously once daily to group 5. Treatment lasted for 28 days.

Induction of diabetes mellitus

Diabetes was induced in overnight fasted rats in the next morning by a single intraperitoneal injection of a freshly prepared solution of 65mg/kg of streptozotocin (STZ) obtained from Sigma Aldrich Chemicals Company, St. Louis, MO, USA in citrate buffer (0.1 M, pH 4.5). Diabetes mellitus was confirmed by fasting blood sugar concentration (≥ 200mg/dl) via tail puncture two days after the induction using a portable glucometer and strips (Accu-Chek, Roche, Germany)[22,23].

Collection of blood samples

After 28 days of treatment, the animals were fasted for 12hours overnight and fasting blood glucose level determined using Accu-chek Glucometer. The animals were anasthesized using chloroform vapour and blood samples collected via cardiac puncture using sterile needles into plane and EDTA sample bottles. The blood samples in plane tubes were then centrifuged at1000rpm for 10 minutes, serum collected and stored for subsequent biochemical analysis of inflammatory biomarkers.

Determination of serum alanine aminotransferase (ALT)

Serum Alanine aminotransferase (ALT), is measured by monitoring the concentration of pyruvate hydrozone formed with 2,4-dinitrophenyhydrozine[24]. The method is based on the principle that pyruvate (pyruvic acid) formed from the alanine aminotransferase catalysed reaction between –ketoglutarate (oxoglutarate) and L-alanine is coupled with chromogen solution (2,4-dinitrophenyl hydrazine) in an alkaline medium to form coloured hydrazone, the concentration of which is proportional to the alanine aminotransferase activity as measured with a colorimeter. To 0.05 ml of each serum sample in a test tube was added 0.25 ml of buffer/substrate solution. This was incubated at 37°C for 30 min in a water bath followed by the addition of 0.25 ml of chromogen solution. The content was mixed and allowed to stand for 20min at room temperature. Then 2.5 ml of sodium hydroxide (0.4 N) was added and mixed. The absorbance was read after 5 min against the blank at 540 nm. The blanks were treated as the samples but without the addition of chromogen solution used to stop all the enzymatic reactions. ALT activity (IU/L) was read off from the standard curve[25].

Determination of serum aspartate aminotransferase (AST)

The determination of the blood serum of Aspartate aminotransferase (AST), is measured by monitoring the concentration of oxaloacetate hydrozone formed with 2,4-dinitrophenylhydrazine[24]. The method is based on the principle that oxaloacetate (oxaloacetic acid) that is formed from the aspartate aminotransferase catalyzed reaction between alpha ketoglutarate and aspartate is coupled with chromogen (2,4-dinitrophenyl hydrazine) in alkaline medium to form colored hydrazone. The concentration of the colored hydrazone is proportional to the aspartate aminotransferase activity and is measured with a colorimeter. To 0.05 ml of each serum sample in a test tube was added 0.25 ml of buffer/substrate solution. The content was incubated at 37°C for 60 min in a water bath followed by the addition of 0.25 ml of chromogen solution. The content was mixed and allowed to stand for 20 min at room temperature after which 2.5 ml of sodium hydroxide (0.4 N) was added and mixed. The absorbance was read after 5 min against blank at 540 nm. The blanks were treated as the samples but without the addition of chromogenic solution used to stop all enzymatic reactions. AST activity (IU/L) was read off from the standard curve[25].

Determination of serum alkaline phosphatase (ALP)

This measurement of alkaline phosphatase (ALP) followed standard procedure[26].

Principle: Phenol released by enzymatic hydrolysis from phenylphosphate under defined conditions of time, temperature and pH – is estimated colorimetrically.

Technique

Test:- 1ml of buffer was mixed with 1ml of phenylphosphate substrate in a test tube placed in water bath at 37°C for 3 minutes. 0.1ml of serum was added mixed gently and incubated for exactly 15 minutes, the reaction was stopped by addition of 0.8ml of 0.5N sodium hydroxide (NaOH). Control:- In a test tube 1ml substrate was mixed with 0.8ml of 0.5N sodium hydroxide, followed by 0.1ml of serum. Standard:- 1.1ml of buffer was mixed with 0.1ml of phenol standard (1mg/100ml) and 0.8ml of 0.5N sodium hydroxide. Blank:- 1.1ml of buffer, 1.0ml of water and 0.8ml of 0.5N sodium hydroxide was mixed. To all tubes 1.2ml of 0.5N sodium bicarbonate ($NaHCO_3$) was added with 1ml of Potassium Ferricyanide solution -$K_3(Fe(CN)_6)$, mixing each tube well after each addition. The successive additions adjusted the pH to develop the color. The 0.0 of reddish –brown colors of 510 nanometer (nM), was read avoiding exposure to strong sunlight.

Calculation:

Serum alkaline phosphatase (King-Armstrong Units/100ml) =

$$\frac{\text{Reading of unknown} - \text{Reading of control}}{\text{Reading of standard} - \text{Reading of Blank}} \text{X } 100$$

Determination of serum sodium and potassium concentration

Flame photometry method was used[27].

Principle:

Potassium or sodium solution under carefully controlled conditions as a very fine spray is supplied to a burner. In the flame the solution evaporates, the salt dissociates to give neutral atoms. Some of these move into a high energy state.

When these excited atoms fall back to the ground state – the list or characteristic wave length emitted – 590nm for sodium and 770nm for potassium. This high passes through a suitable filter on to photosensitive element and the amount of current thus produced is measure.

Determination of chloride ion concentration

This was determined using the standard method[28]. A volume of 0.2ml of serum was placed in a universal container. 2ml of distilled water was added, and two drops of diphenyl carbazone indicator was also added. To the solution, a drop of 2N nitric acid was added and the mixture vigorously mixed.

Determination of Bicarbonate

The back titration method was used to estimate bicarbonate[29]. Briefly, excess standardized dilute H_2SO_4 was added to the serum. The CO_2 emitted by HCO_3 was calculated as an equivalent amount of H^+ removed for water formation (H_2O). Using natural red as an indicator, excess standardized H_2SO_4 was titrated against 0.01N NaOH. The endpoint was indicated by a pink color. The titrimetric mercuric nitrate method was used to determine the chloride concentration in serum.

Determination of plasma Creatinine and Urea

Creatinine and urea concentrations for renal function were determined using a Randox test kit following standard protocol[24,30]

Data Analysis

Results are expressed as mean ± SEM. Data was analyzed using the GraphPad Prism software (version 6.0). Analysis of variance (ANOVA) followed by Turkey comparison test where F value was significant. Probability level of $p<0.05$ was accepted as significant.

RESULTS

Effect of GL on serum Sodium (Na^+) level

The mean serum sodium concentration in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 142 ±0.63 mmol/L, 139 ±0.39 mmol/L, 145 ±0.30 mmol/L, 143 ±0.46 mmol/L. and 151 ±0.97 mmol/L, respectively. The result shows a significant ($p<0.05$) increased Na^+ in the DM group compared with control. But treatment with GL reduced it towards normal. This is shown in figure 1.

Effect of GL on Potassium ion (K^+) level

The mean serum potassium in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 3.5 ±0.12 mmol/L, 4.4 ±0.057 mmol/L, 4.9 ±0.17 mmol/L, 4.0 ±0.071 mmol/L. and 6.5 ±0.075 mmol/L, respectively. The result shows a significant ($p<0.05$) increased K^+ in the DM group compared with control. Treatment with GL reduced it towards normal. This is presented in figure 2.

Effect of GL on Serum chloride level

The mean serum chloride in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 102 ±0.70 mmol/L, 105 ±0.57 mmol/L, 106 ±0.47 mmol/L, 104 ±0.47 mmol/L and 118 ±0.69 mmol/L, respectively. The result shows a significant ($p<0.05$) increased Cl^- in the DM group compared with control. Treatment with GL significantly ($p<0.005$) reduced it towards normal, as presented in figure 3.

Effect of GL on Bicarbonate level

The mean serum bicarbonate in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 23 ±0.29 mmol/L, 21 ±0.31 mmol/L, 21 ±0.34 mmol/L, 22 ±0.59 mmol/L and 18 ±0.39 mmol/L respectively. The result shows a significant ($p<0.05$) decreased Hco3 in the DM group compared with control. Treatment with GL increase it towards normal, see figure 4

Effect of GL on serum Urea

The mean serum urea in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 5.6 ±0.099 mmol/L, 4.9 ±0.12 mmol/L, 9.4 ±0.13 mmol/L, 5.1 ±0.087 mmol/L and 11 ±0.35 mmol/L, respectively. The result shows a significant ($p<0.05$) increased urea in the DM group compared with control. GL decreased serum urea back to normal values, figure 5.

Effect of GL on Serum creatinine levels

The mean serum creatinine level in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 114 ±2.7 mg/dl, 104 ±1.8 mg/dl, 125 ±3.0 mg/dl, 108 ±0.48 mg/dl and 160 ±0.86 mg/dl, respectively. The result shows a significant ($p<0.05$) increased creatinine in the DM group compared with the control. Treatment with GL decreased it towards normal. This is presented in figure 6

Effect of GL on serum Alkaline Phosphatase

The mean serum ALP level in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 82 ±2.3 IU/L, 85 ±0.94 IU/L, 92 ±2.0 IU/L, 88 ±1.5 IU/L and 99 ±1.2 IU/L respectively. The result shows a significant ($p<0.01$) increased ALP concentration in the DM group compared with control. Treatment with GL decreased it towards normal, see figure 7.

Effect of GL on serum Alanine aminotransferase

The mean serum ALT level in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 46 ±0.45 IU/L, 36 ±0.73 IU/L, 41 ±0.93 IU/L, 41 ±0.48 IU/L. and 72 ±1.5 IU/L, respectively. The result shows a significant ($p<0.01$) increased ALT concentration in the DM group compared with control. Treatment with GL decreased it towards normal, figure 8.

Effect of GL on serum aspartate aminotransferase (AST)

The mean serum AST level in the control, GL only, DM + GL, DM + Insulin, and DM only groups was 90 ± 0.90 IU/L, 66 ± 0.96 IU/L, 99 ± 0.65 IU/L, 102 ± 0.72 IU/L and 104 ± 1.4 IU/L, respectively. The result shows a significant ($p<0.01$) increased AST concentration in the DM group compared with control. GL decreased it towards normal, figure 9.

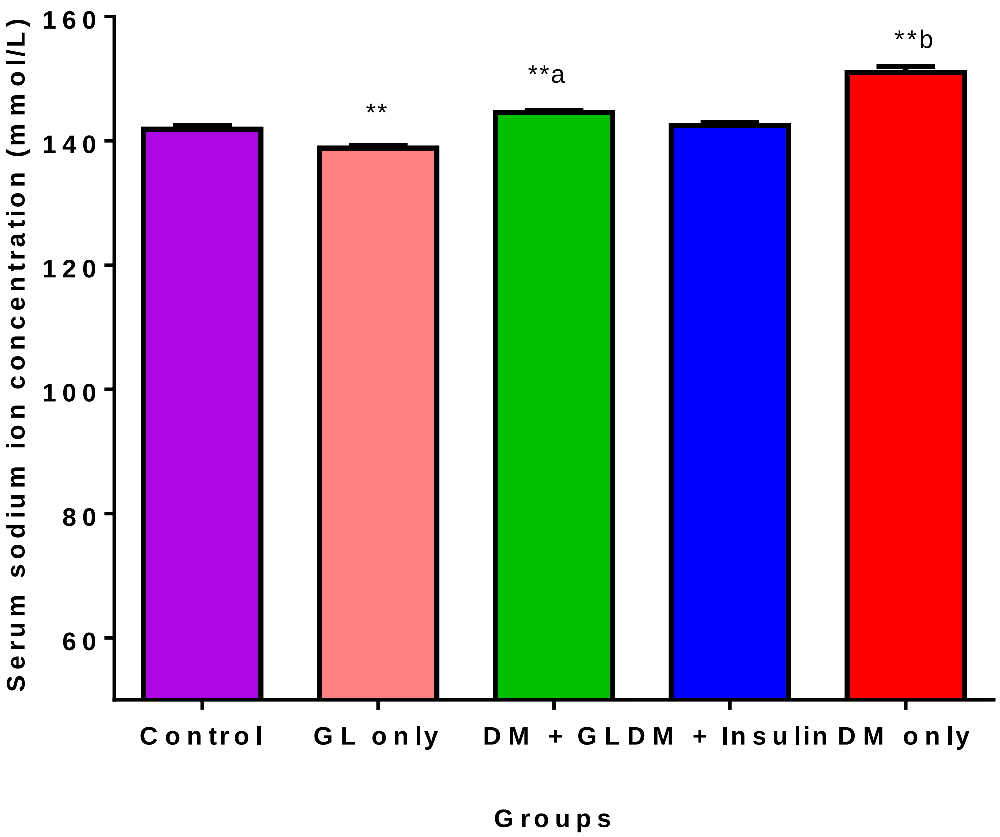

Figure 1: Comparison of Serum sodium concentration in the experimental groups

**=p<0.01 compared with control; a = p<0.01 versus GL only group; b = p<0.01 versus DM+insulin group

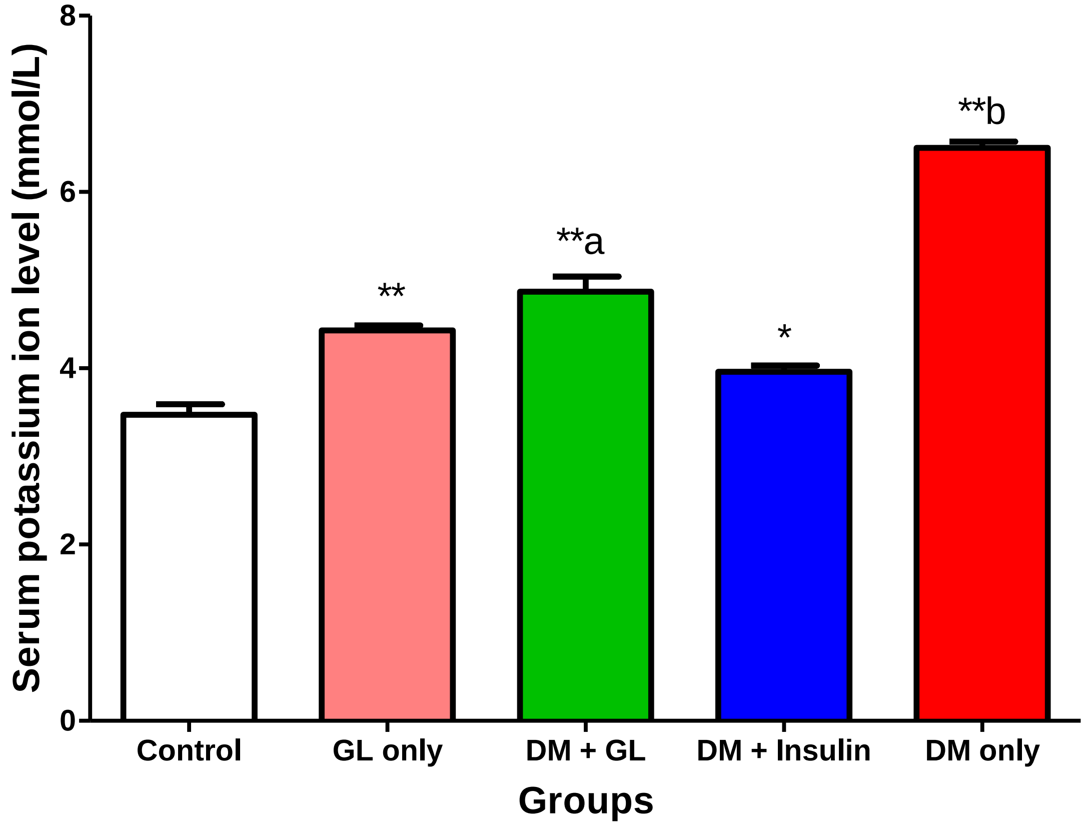

Figure 2: Comparison of serum potassium ion level in the experimented groups

** =p<0.01 compared with control; * =p<0.05 compared with control; a =p<0.01 compared with GL only group; b =p<0.01 compared with DM+insulin.

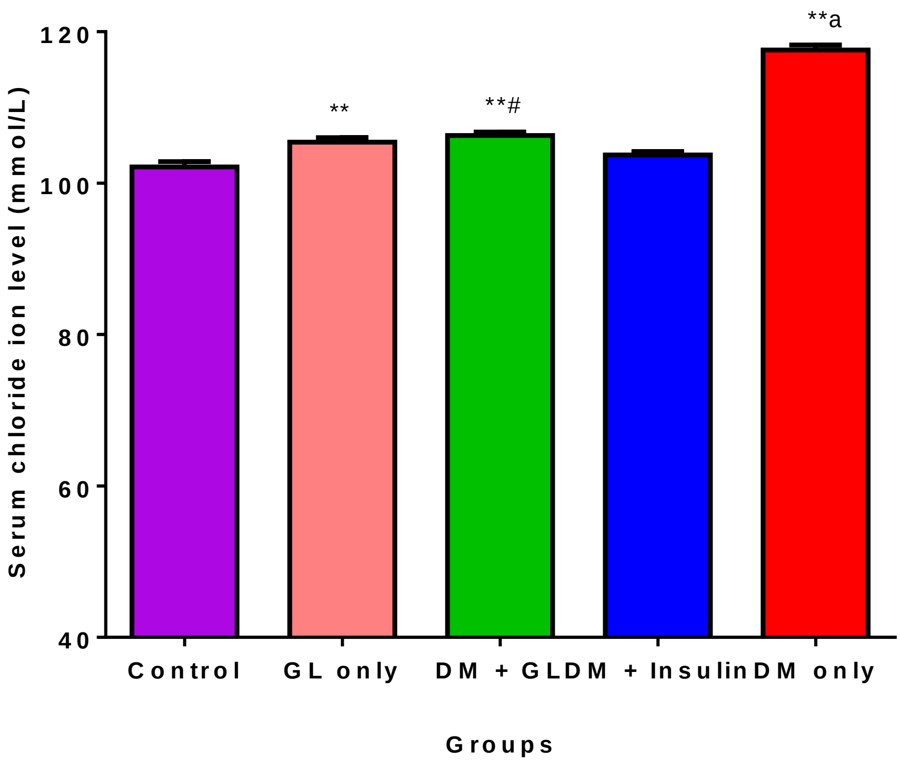

Figure 3: Comparison of serum chloride ion level in the experimental groups

** = p<0.01 versus control; # = p<0.05 versus DM + insulin

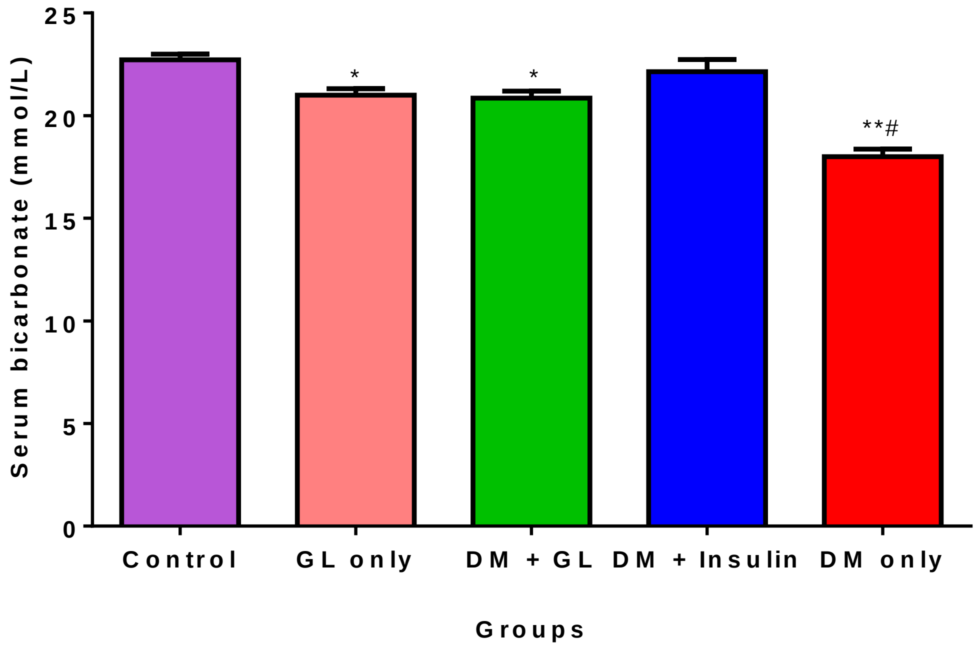

Figure 4: Comparison of serum bicarbonate ion levels in the experimental groups

* = p<0.05 compared with control; # = p<0.01 compared with DM+ GL and DM + insulin groups

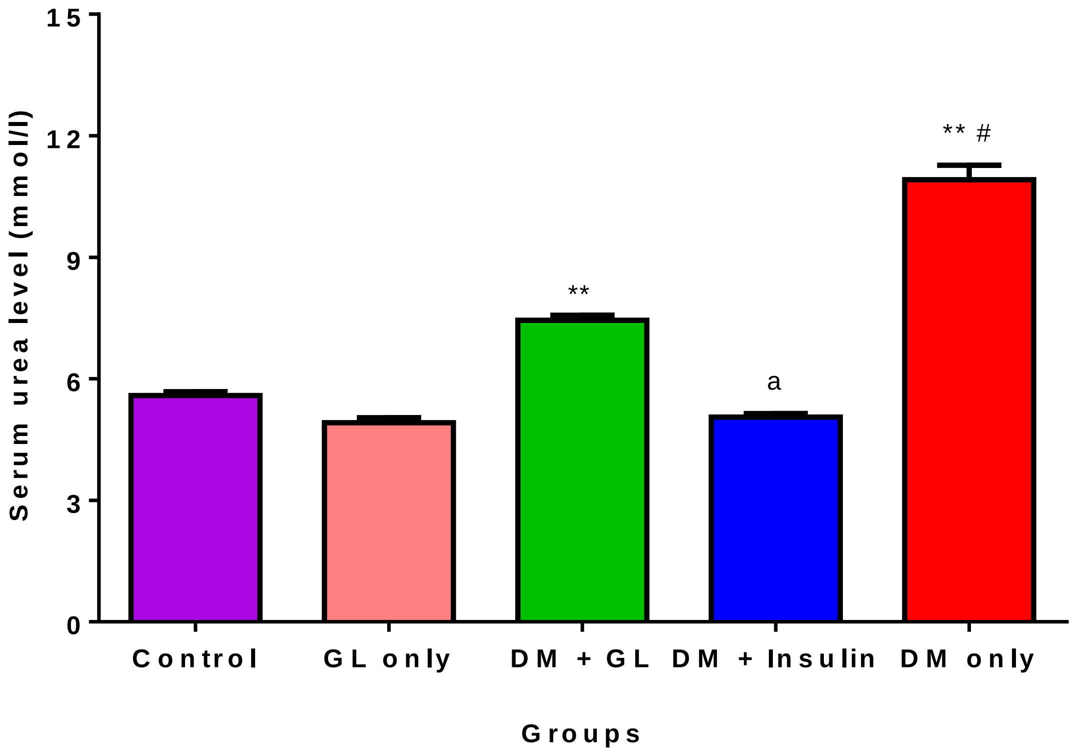

Figure 5: Comparison of serum urea level in the experimental groups

* = p<0.05 compared with control; # = p<0.01 compared with DM+ GL and DM + insulin groups . a =p<0.01 compared with GL only group

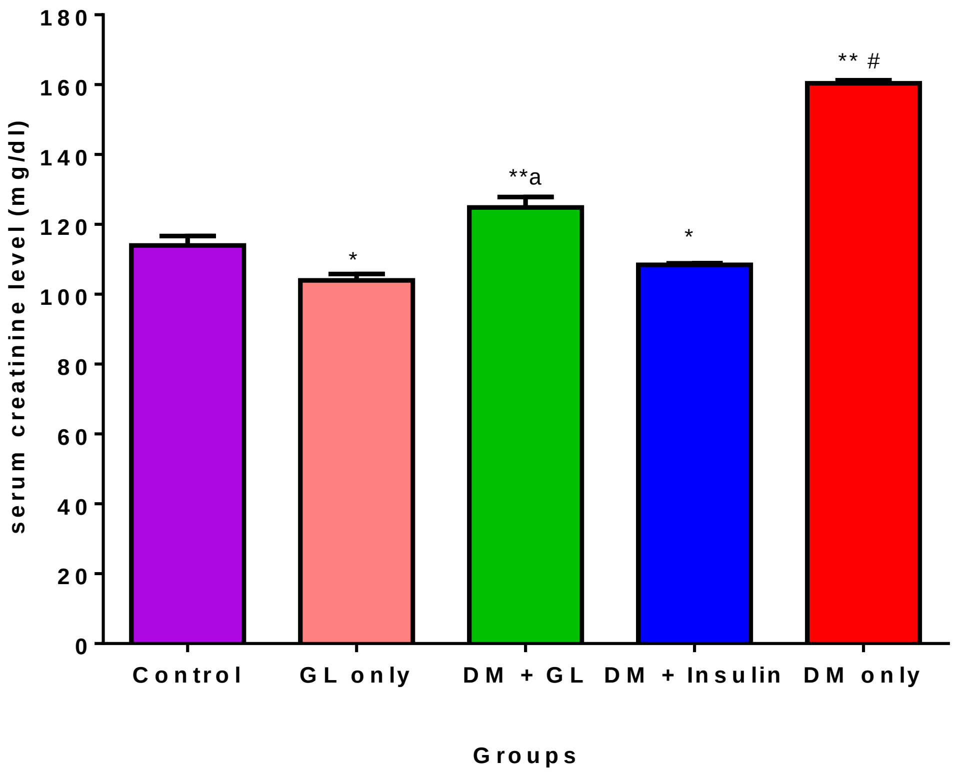

Figure 6: Comparison of serum creatinine level in experimental groups

* = p<0.05 compared with control; # = p<0.01 compared with DM+ GL and DM + insulin groups . a =p<0.01 compared with GL only group

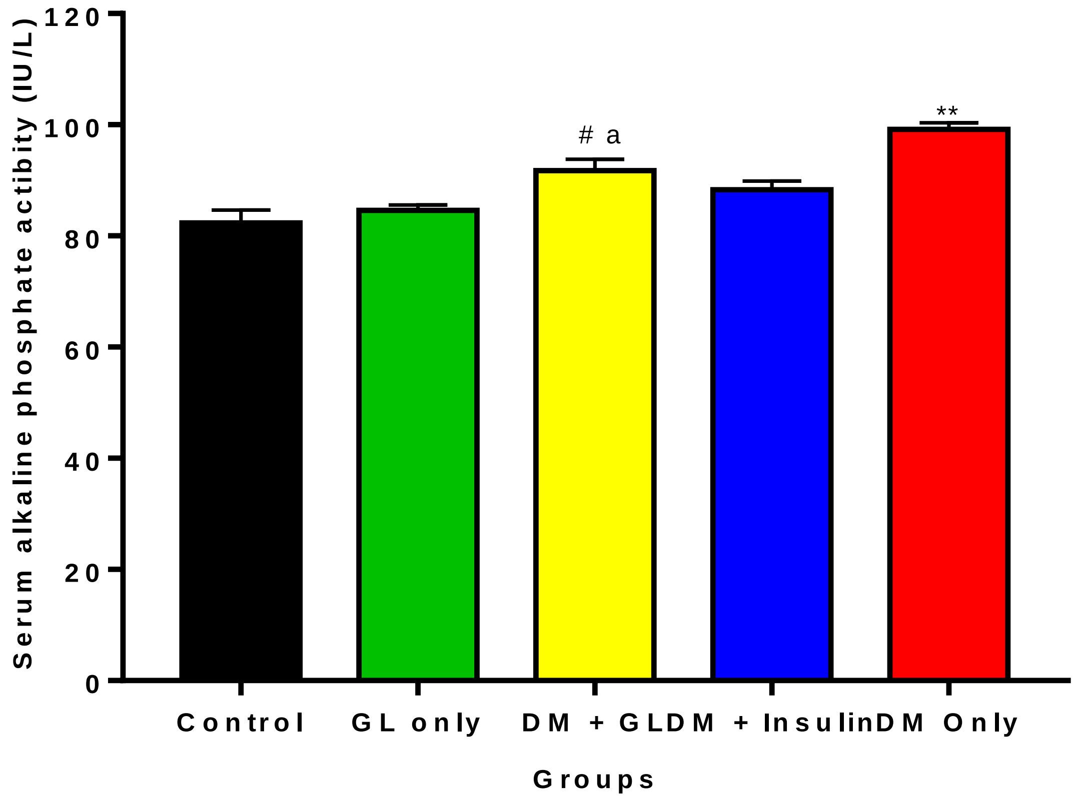

Figure 7: Comparison of serum alkaline phosphatase activity in the experimental group

** = p<0.05 compared with control; # = p<0.01 compared with DM+ GL and DM + insulin groups . a =p<0.01 compared with GL only group.

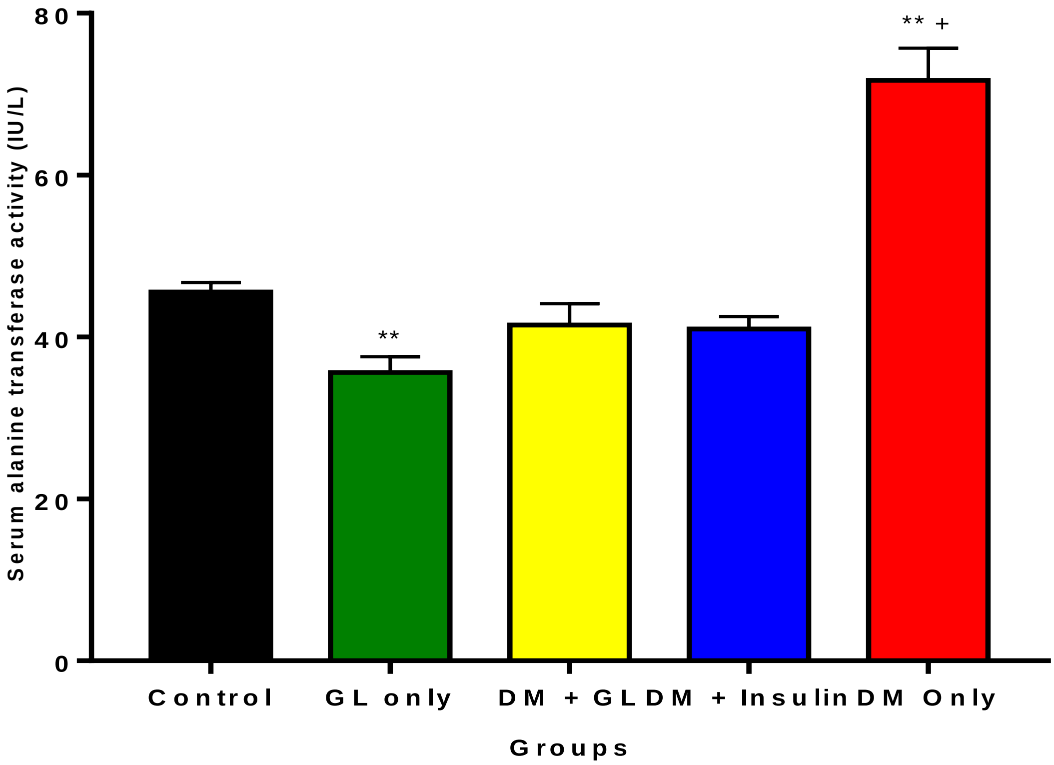

Figure 8: Comparison of serum alanine aminotransferase in the diabetic group

** = p<0.05 compared with control; **+ = p<0.01 compared with DM+ GL and DM + insulin groups.

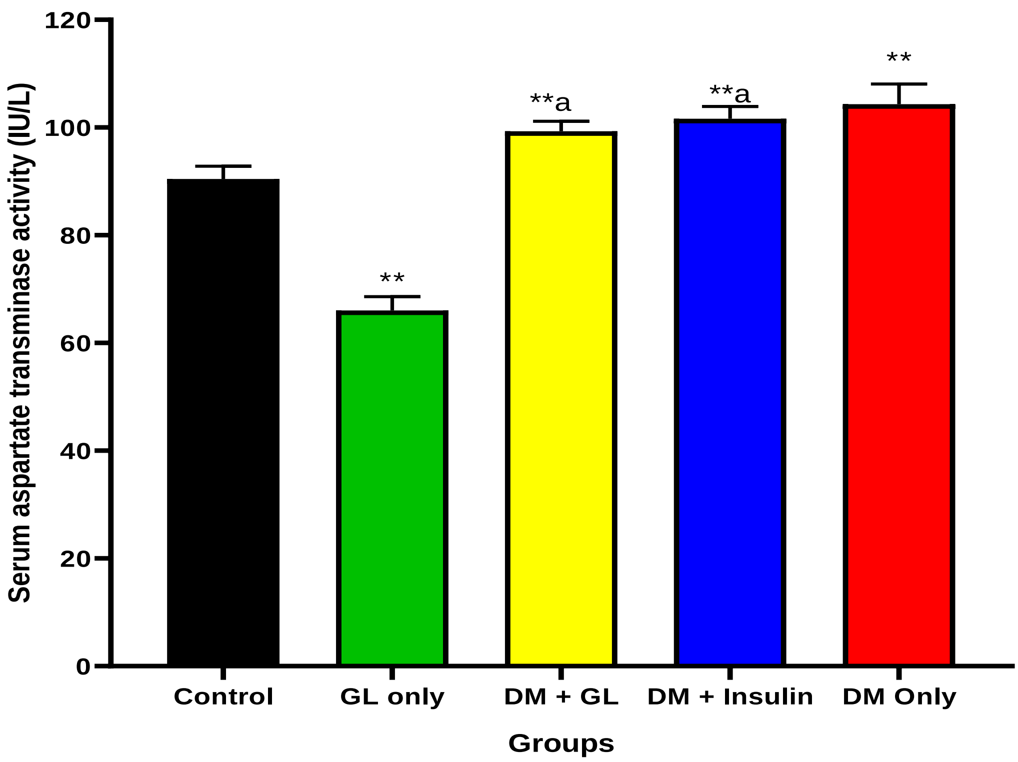

Figure 9: Comparison of serum aspartate transaminase activity in the experimental groups

** = p<0.05 compared with control; # = p<0.01 compared with DM+ GL and DM + insulin groups . a =p<0.01 compared with GL only group

DISCUSSION

This study was aimed at investigating the effect of ethanolic leaf extract of *Gongronema latifolium* on serum electrolytes and liver enzymes activities in streptozotocin-induced diabetic rats. Also, the effect of the extract on blood glucose levels of rats treated with *G.latifolium* and insulin were also determined.

Electrolytes are minerals that regulate the homeostatic functions of the body and help to maintain osmotic equilibrium between the intracellular and extracellular fluids[31]. In a disease condition such as DM, the body's electrolyte control system are altered. Also, plasma electrolytes and metabolites (such as creatinine, urea and blood urea nitrogen) are used to assess kidney functions[32]. The result presented above showed no significant changes in serum Na^+, K^+, Cl^- and bicarbonate concentration when comparing DM+GL with DM+insulin treated groups. This decrease in Na^+, K^+, Cl^- in the two treated groups may be due to the presence of tannins that may have regulated their bioavailability of these electrolytes[33,34,35]. The effect of extract on electrolytes in diabetic treated animals indicated nephron-protective potential of the plant extract. This is in agreement with the report by Akpanabiatu[36]. that reported beneficial effect of *Gongronema latifolium* on electrolyte levels in diabetic condition.

Creatinine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle for energy production. Creatinine retention in the blood is evidence of kidney impairment[37]. The decrease in serum creatinine and urea level in *Gongronema latifolium*-treated rats when compared with the DM only group indicates that this plant may also possess hepato-protective function.

Hepatocellular injury whether acute or chronic, results in an increase in serum concentrations of some liver enzymes such as alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and aspartate aminotranferase (AST). These enzymes are useful in detecting hepatic disease conditions. The result presented above shows an increase in ALP, ALT and AST in DM only group which is an indication of necrosis and compromised integrity of liver cell membranes[38]. The reduced activities of ALP, ALT and AST in *G. latifolium* treated rats (DM + GL) compared to insulin treated rats (DM + insulin) could be attributed to the ability of *G. latifolium* to prevent hepatic damage. It may have also initiated the healing and regeneration of liver parenchyma and cells, respectively which shows that the plant has hepatoprotective effects[39]. Therefore, base on the findings and the results obtained it was concluded that Gongronema latifolium has nephroprotective and hepatoprotective ability during diabetic condition.

In conclusion, treatment and management of metabolic disorder such as diabetes has gain global attention and thus require immediate intervention. The use of medicinal plants in managing these diseases requires proper scientific measures. This study revealed that administration of *Gongronema latifolium* leave extracts to diabetic rats normalized electrolytes balance and reduced liver damage which reveals that the plant possess nephro and hepato-protective properties.

ACKNOWLEDGEMENT

The authors of this manuscript wish to heartily acknowledge the following persons for their valuable contributions to the success of this research work and subsequent publication of the manuscript. Mr. Ededet Umoh of the Department of Physiology, University of Calabar, for his assistance to feed, sacrifice and conduct the collection of blood from the animals. Dr. Iya Eze Bassey of the Department of Chemical Pathology, University of Calabar Teaching Hospital, for carrying out the different biochemical assays in this study.

Conflict of interest:

None of the authors have a conflict of interest

Ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences University of Calabar Animal Research and Ethical Committee with ethical number: (019PY20317).

Informed consent

Informed consent was obtained from all individual participants included in the study

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care 2013; 36:(Suppl 1):S67-74. https://doi.org/10.2337/dc13-S067

2. Kumar S, Behl T, Sachdeva M, Sehgal A, Kumari S, Kumar A, Kaur G, Yadav HN, Bungau S. Implicating the effect of ketogenic diet as a preventive measure to obesity and diabetes mellitus. Life Sci 2021; 264:118661. https://doi.org/10.1016/j.lfs.2020.118661

3. Maher A, Jermeen EY, Manar SM, Amira H. Effect of Ultrasound Cavitation on Weight Reduction for Prediabetic Obese Patients. The Medical Journal of Cairo University 2019; 87: 909-917 https://doi.org/10.21608/mjcu.2019.52713

4. Barron E, Bakhai C, Kar P, Weaver A, Bradley D, Ismail H, Knighton P, Holman N, Khunti K, Sattar N, Wareham NJ. Associations of type 1 and type 2 diabetes with COVID-19-related mortality in England: a whole-population study. The Lancet Diab Endocrin 2020; 8(10):813-822. https://doi.org/10.1016/S2213-8587(20)30272-2

5. Chan JC, Lim LL, Wareham NJ, Shaw, JE, Orchard TJ, Zhang P, Lau ES, Eliasson B, Kong AP, Ezzati M, Gregg EW. The Lancet Commission on diabetes: using data to transform diabetes care and patient lives. The Lancet 2021; 396(10267): 2019-2082. https://doi.org/10.1016/S0140-6736(20)32374-6

6. Chen H, Li R. Introduction of Diabetes Mellitus and Future Prospects of Natural Products on Diabetes Mellitus. In Structure and Health Effects of Natural Products on Diabetes Mellitus, Springer, Singapore, 2021; 1-15. https://doi.org/10.1007/978-981-15-8791-7_1

7. Chung SS, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. J Ame Soc Nephrol 2003; 14(suppl 3): S233-S236. https://doi.org/10.1097/01.ASN.0000077408.15865.06

8. Mora C, Navarro JF. Inflammation and diabetic nephropathy. Current Diabetes Reports 2006; 6(6):463-468. https://doi.org/10.1007/s11892-006-0080-1

9. Green ME, Shah BR, Slater M, Khan S, Jones CR, Walker JD. Monitoring, treatment and control of blood glucose and lipids in Ontario First Nations people with diabetes. CMAJ 2020; 192(33): E937-E945. https://doi.org/10.1503/cmaj.191039

10. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27(5):1047-1053. https://doi.org/10.2337/diacare.27.5.1047

11. Okpala JC, Igwe JC, Ifedilichukwu HN. Effects of N-Butanol fraction of Gongronema Latifolium leave extract on some liver function and histological parameters in CCl4-induced oxidative damage in wistar albino rats. Biochem Pharm 2014a; 3(126):2167-2171. https://doi.org/10.4172/2167-0501.1000126

12. Okpala JC, Sani I, Abdullahi R, Ifedilichukwu HN, Igwe JC. Effects of n-butanol fraction of Gongronema latifolium leave extract on some biochemical parameters in CCl4-induced oxidative damage in Wistar albino rats. Afr J Biochem Res 2014b; 8(2):52-64. https://doi.org/10.5897/AJBR2013.0741

13. Ugochukwu NH. Babady NE. Antioxidant effects of Gongronema Latifolium in hepatocytes of rat models of non-insulin dependent diabetes mellitus. Fitoterapia 2002; 73(7):612-618. https://doi.org/10.1016/S0367-326X(02)00218-6

14. Constance N, Joy DN, Nkeiruka OA, Ahamefula E, Chidimma A, Oluchi AA. Protective Effects of Bi-Herbal Formulation of Aqueous Extracts of Vernonia amygdalina and Gongronema latifolium against Gentamicin Induced Nephrotoxicity and Liver Injury in Rats. Asian J Res Biochem 2020; 7(4):12-20. https://doi.org/10.9734/ajrb/2020/v7i430144

15. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR The effect of Gongronema Latifolium leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. J BioscI 2003; 28:2-4. https://doi.org/10.1007/BF02970124

16. Morebise O. (2015). A review on Gongronema Latifolium, an extremely useful plant with great prospects. Eur J Med Plants 2015; 10(1):1-9. https://doi.org/10.9734/EJMP/2015/19713

17. Ojiako OA, Chikezie PC. Blood Na+/K+ and Cl-Levels of Hyperglycemic Rats Administered with Traditional Herbal Formulations. Pharmacogn Commun 2015; 5:140-145. https://doi.org/10.5530/pc.2015.2.5

18. Hossain MA, Mostofa M, Debnath D, Alam AK Yasmin Z, Moitry NF. Antihyperglycemic and antihyperlipidemic of Karala (Momordica charantia) fruits in streptozotocin induced diabetic rats. J Environ Sci Nat Res 2012; 5(1):29-37. https://doi.org/10.3329/jesnr.v5i1.11550

19. Javed F, Al-Askar M, Al-Hezaimi K. Cytokine profile in the gingival crevicular fluid of periodontitis patients with and without type 2 diabetes: a literature review. J Periodontology 2012; 83(2):156-161. https://doi.org/10.1902/jop.2011.110207

20. Schindhelm RK, Diamant M, Heine RJ. Nonalcoholic fatty liver disease and cardiovascular disease risk. Current Diabetes Reports 2007; 7(3):181-187. https://doi.org/10.1007/s11892-007-0030-6

21. Mathur S, Mehta DK, Kapoor S, Yadav S. Liver function in type-2 diabetes mellitus patients. Int J Sci Study, 2016; 3(10):43-47.

22. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, Eberhardt NL, Kudva YC. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Lab animals 2012; 45(3):131-140. https://doi.org/10.1258/la.2010.010090

23. Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J Diab Metc Dis 2013; 12(1):60. https://doi.org/10.1186/2251-6581-12-60

24. Reitman S, Frankel S. Determination of aminotransaminases in serum. Ame J Clin Path 1957; 2(8):50-56. https://doi.org/10.1093/ajcp/28.1.56

25. Egbuonu ACC, Obidoa O, Ezeokonkwo CA, Ejikeme PM. Hepatotoxic effects of low dose oral administration of monosodium glutamate in male albino rats. Afri J Biotechn: 2009; 8(13):3031-3035. https://doi.org/10.4314/br.v7i1.45475

26. King EJ, Armstrong AR. Can Med Assoc J 1964; 31:376 https://doi.org/10.1007/BF02762226

27. Harris, D. C. (1995). Quantitative Chemical Analysis 4th Ed., New York. Freeman, W. H and Company.

28. Schales O, Schales. SS. A simple and accurate method for the determination of chloride in biological fluids. J Bioll Chem 1941; 140:879-884. https://doi.org/10.1016/S0021-9258(18)72872-X

29. Anrade DE. Determination of bicarbonate using back titration. Am. Chem. Soc, 1995; 35:49-54.

30. Asiwe JN, Daubry TME, Okon IA, Akpotu AE, Adagbada EO, Eruotor H, Agbugba LC, Buduburisi BR. Ginkgo biloba Supplement Reverses Lead (II) Acetate-Induced Haematological Imbalances, Hepatic and Renal Dysfunctions in Male Wistar Rat. Biol Trace Element Res 2022; 1-11. https://doi.org/10.1007/s12011-022-03098-6

31. Rhoda KM, Porter MJ, Quintini C. Fluid and electrolyte management: putting a plan in motion. J Parenteral Enteral Nutri 2011; 35(6):675-685. https://doi.org/10.1177/0148607111421913

32. Usoh IF, Akpan HD, Akpanyung EO. Nephroprotection against streptozotocin diabetes is more effective in combined than single leaves extracts of Gongronema Latifolium and Ocimum Gratissimum L. J nnovations Pharma Biol Sci 2016; 3(1):001-016.

33. Akinsola, A. F., Osasona, I., Akintayo, E. T., Siyanbola, T. O., & Omosebi, S. O. Nutritional Evaluation of Calabash Gourd (L agenaria Siceraria) Seeds and Oil. Journal of Culinary Science & Technology, 2022; 1-22. https://doi.org/10.1080/15428052.2021.2016527

34. Egbung GE, Essien NA, Mgbang JE, Egbung JE. Serum lipid and electrolyte profiles of Wistar rats fed with Vernonia amygdalina supplemented Vigna subterranea (Bambara groundnut) pudding. Calabar J Health Sci 2020; 3(2):40-45. https://doi.org/10.25259/CJHS_10_2019

35. Eneji EE, Atangwho IJ, Iwara IA, Eyong UE. Micronutrient and phyto-chemical composition of root bark and twig extracts of Gongronema Latifolium. J Med Med Sci 2011; 2(11):1185-1188.

36. Akpanabiatu MI, Umoh IB, Udosen EO, Udoh AE, Edet EE. Rat serum electrolytes, lipid profile and cardiovascular activity on Nauclea latifolia leaf extract administration. . Indian J Clin Biochem 2005b; 20(2):29-34. https://doi.org/10.1007/BF02867397

37. Imo C, Friday OU. Renal Protective Effect of Ethanolic Leaf Extract of Gongronema Latifolium Benth in Acetaminophen-induced Renal Toxicity in Male Albino Rats. Am Chem Sci J 2015; 8(3):1-10. https://doi.org/10.9734/ACSJ/2015/18881

38. Thapa BR, Walia A. Liver function tests and their interpretation. The Indian J Pediatrics 2007; 74(7):663-671. https://doi.org/10.1007/s12098-007-0118-7

39. Ajiboye BO, Oyinloye BE, Udebor EA, Owolabi OV, Ejeje JN, Onikanni SA, Omotuyi OI. Hepatoprotective potential of flavonoid-rich extracts from Gongronema latifolium benth leaf in type 2 diabetic rats via fetuin-A and tumor necrosis factor-alpha. Mol Biol Rep 2022; 1-10. https://doi.org/10.1007/s11033-022-07657-x