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

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**INTRODUCTION**

Diabetes mellitus (DM) is a pathological and metabolic condition characterized by impaired glucose metabolism caused by inadequate insulin action or insulin resistance. Clinically, it is defined as a fasting plasma glucose level > 7.8 mmol/l (140mg/dl) or a 2-hour post-prandial plasma glucose > 11 mmol/l (200 mg/dl). 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. It is a complicated and chronic disease with complex etiologies, 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.

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. 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.

*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. 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, 'utazji' by the Igbos and arooke by the Yorubas. 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 and also reduces renal and hepatic oxidative stress, lipid peroxidation, and increases the glutathione/glutathione disulphide (GSH/GSSG) ratio. *G. latifolium* crude leaf extract is used in the treatment of malaria, diabetes, hypertension, and as laxative.
Electrolyte imbalance is markedly present in patients 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. Several studies have estimated the electrolytes levels in diabetes mellitus in several countries and showed the association between electrolytes and hyperglycemia.

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. 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. 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 aimed at investigating the hepatoprotective and nephroprotective effect of Gongronema latifolium leaf extract in Streptozotocin Induced diabetic rat.

The aim of this study was 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. 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 rotary 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. 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).

**Collection of blood samples**

After 28 days of treatment, the animals were fasted for 12hours overnight and fasting blood glucose level determined using Accu-check Glucometer. The animals were anaesthetized using chloroform vapour and blood samples collected via cardiac puncture using sterile needles into plane and EDTA sample bottles. The blood samples in plain 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. 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 hydradine) in an alkaline medium to form coloured hydrozone, 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 20 min 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 hydrony formed with 2,4-dinitrophenylhydrazine. 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 (NaHCO3) was added with 1ml of Potassium Ferricyanide solution -K3(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:**

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\text{Serum alkaline phosphatase (King-Armstrong Units/100ml)} = \frac{\text{Reading of unknown} - \text{Reading of control}}{\text{Reading of standard} - \text{Reading of Blank}} \times 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 – 590 nm for sodium and 770 nm 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. A volume of 0.2 ml of serum was placed in a universal container. 2 ml 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. Briefly, excess standardized dilute H2SO4 was added to the serum. The CO2 emitted by HCO3 was calculated as an equivalent amount of H+ removed for water formation (H2O). Using natural red as an indicator, excess standardized H2SO4 was titrated against 0.01 N 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 ±0.07 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.

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**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<0.05 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 activity 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 fluids31. 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 functions32. 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 serum creatinine and urea level in Gongronema latifolium-treated rats when compared with the DM only group indicates that this plant may also possess hepatoprotective 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 aminotransferase (AST). These enzymes are useful in detecting hepatic disease conditions. The result presented above shows an increase in ALT, AST and AST in DM only group which is an indication of necrosis and compromised integrity of liver cell membranes36. The reduced activities of ALT, AST 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 effects37. 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 leaf extract to diabetic rats normalized electrolytes balance and reduced liver damage which reveals that the plant possess nephro and hepatoprotective properties.

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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

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