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HYPOLIPIDEMIC AND PLASMA PROTEINS STABILIZING EFFECT OF GONGRONEMA LATIFOLIUM IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objectives: *Gongronema latifolium* (GL) is a medical plant with myriad healing potentials. But it's effect on serum lipids and proteins in diabetic condition has net been elucidated. This work was therefore designed to evaluate the effect of ethanolic leaf extract of *Gongronema latifolium* on lipid profile and plasma proteins in Streptozotocin-induced diabetic rats. **Methods:** A total of 40 Wistar rats of both sexes initially weighing between 150g-200g were divided into five (5) groups of eight (8) rats each. Group 1 was the normal control (administered 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 days with an interval of one day in between, their blood was collected via the tail vein for glucose determination using a portable glucometer. Group 4 was the diabetic + 200mg/kg GL group. Group 5 was the diabetic + insulin (subcutaneously). The feeding regimens lasted 28 days. Blood samples were collected via cardiac puncture. **Results:** There was 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. High density lipoprotein was significantly ($p<0.01$) decreased in the diabetic group with a corresponding increase in total cholesterol, low density lipoprotein, triglycerides, and very low lipoprotein. Treatment with GL and insulin reversed the changes towards normal. There is also a decrease in total serum protein in *Gongronema latifolium* treated group when compared with the insulin treated group, also a corresponding significant decrease in globulin and albumin level in the GL+DM treated group compared with the diabetic group. **Conclusion:** *Gongronema latifolium* leaf extract has an hypolipidemic potential and also stabilizes plasma proteins levels in diabetic condition and target organ.

KEYWORD: *Gongronema Latifolium*, Diabetes Mellitus, serum lipids, Proteins, STZ, insulin.

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 2 hour 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). Diabetes mellitus is generally 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] which can lead to reduced glucose tolerance, nerve damage, kidney failure, atherosclerosis, stroke, blindness and heart disease.

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.[6] 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.[7]

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.[8] The leafy vegetable can be propagated by seed. Its common name is 'amaranth

globe'. In Nigeria, *G. latifolium* is known by different local names. In Nigeria, it has different names such as 'utasi' by the Efiks/Ibibios, '*utazi*' by the Igbos and *arokeke* by the Yorubas.[9] *G. latifolium* crude leaf extract is used in the treatment of malaria, diabetes, hypertension, and as laxative.[10]

It possesses antioxidant activity by increasing superoxide dismutase and glutathione peroxidase activities[11] and also reduces renal and hepatic oxidative stress, lipid peroxidation, and increases the glutathione/glutathione disulphide (GSH/GSSG) ratio.[9,12]

Lipids and lipoproteins are risk factors for CHD. It has been demonstrated that high levels of serum total cholesterol (TC), triglycerides (TG), LDL cholesterol, very-low-density lipoprotein (VLDL), low concentration of HDL cholesterol, and increased body mass index (BMI) are significantly associated with CHD (George and Ludvik 2000). Dyslipidemia is one of the top five major risk factors leading to cardiovascular disorders. It is characterized by elevated LDL cholesterol and TG and decreased HDL cholesterol. Cholesterol is oxidized by the liver into a variety of bile acids.[13] These, in turn, are conjugated with glycine, taurine, glucuronic acid, or sulfate. A mixture of conjugated and nonconjugated bile acids, along with cholesterol itself, is excreted from the liver into the bile. Approximately 95% of the bile acids are reabsorbed from the intestines, and the remainder are lost in the feces.[14] The excretion and reabsorption of bile acids forms the basis of the enterohepatic circulation, which is essential for the digestion and absorption of dietary fats. Under certain circumstances, when more concentrated, as in the gallbladder, cholesterol crystallizes and is the major constituent of most gallstones (lecithin and bilirubin gallstones also occur, but less frequently.[15] Every day, up to 1 g of cholesterol enters the colon. This cholesterol originates from the diet, bile, and desquamated intestinal cells, and can be metabolized by the colonic bacteria. Cholesterol is converted mainly into coprostanol, a nonabsorbable sterol that is excreted in the feces. A cholesterol-reducing bacterium origin has been isolated from human feces.[16]

Serum proteins are produced by hepatocytes in the liver in response to pro-inflammatory cytokines (tumour necrosis factor-alpha, interleukin-1 and interleukin-6). Their synthesis constitutes a normal response to tissue inflammation or injury. These reactant helps in minimizing local tissues damage and participate in tissue repair, as well as aiding microbial killing.[17]

The globulins are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Some globulins are produced in the liver, while others are made by the immune system. Globulins, albumins, and fibrinogen are the major blood proteins. The normal concentration of globulins in human blood is about 2.6-4.6 g/dL. Globulins are distinguished from one another using serum protein electrophoresis and exist in various sizes.[17]

Serum albumin often referred to simply as blood albumin, is an albumin (a type of globular protein) found in vertebrate blood. Human serum albumin is encoded by the ALB gene. Albumin is essential for maintaining the oncotic pressure needed for proper distribution of body fluids between blood vessels and body tissues; without albumin, the high pressure in the blood vessels would force more fluids out into the tissues. It also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones and as a transport protein for hemin and fatty acids. Too much or too little circulating serum albumin may be harmful. Excess serum albumin and its presence in urine usually denotes the presence of kidney disease. However, occasionally albumin appears in the urine of normal persons following long standing (postural albuminuria).[17]

MATERIALS AND METHODS

Experiment animal

Forty adult Wistar rats of both sexes equal number weighing 150-200 g were used for the study. The animals were purchased from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH), Okuku Campus, Cross River State, Nigeria. The animals were kept under experimentally controlled conditions (27±2°C, with 12hours light-dark cycle). All the animals were acclimatized for 1week before the commencement of the experiment. They were kept in plastic cages and fed with normal rat chow and tap water *ad libitum*. Ethical approval was obtained from the Faculty of Basic Medical Sciences University of Calabar Animal Research and Ethical Committee with ethical number No: 019PY20317).

Preparation of *Gongronema latifolium* extract

The preparation of extract was according to standard method.[9] *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.

Experimental design

The Wistar rats were divided into five groups equal no of both sexes of eight rats each and were placed in different rat cages for proper identification. The rats were acclimatized for one week and grouping is shown below.

Group 1: Control
Group 2: STZ Diabetic (DM)
Group 3: *Gongronema latifolium* (GL)
Group 4: DM+GL
Group 5: DM + Insulin

Administration of *Gongronema latifolium* extract and insulin

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. The dosage of plant extracts was administered according to standard method.[9] 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) at a dosage of 65 mg/kg body weight. 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-chek Glucometer. The animals were anaesthetized using 5% chloroform 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 Total Cholesterol

A volume of 0.05ml of serum was mixed with 5ml of ethanol and centrifuged. 3ml of the supernatant was then mixed with 3ml of colour reagent. 3ml of the working standard solution was also mixed with 2ml of the colour reagent. Each tube stayed for 30 minutes at room temperature for maximum colour development. Reading was done using the colorimeter at 50nm wavelength against the reagent blank. Total cholesterol was determined by the calculation[18]:

$$\text{Cholesterol} = \frac{\text{Test} \times 5.2\text{mmol}}{\text{Standard}}$$

Determination of HDL-cholesterol

A volume of 0.2ml of $MnCl_2$-heparin reagent was mixed with 0.2ml of serum in a test tube. This was to precipitate all lipoproteins in the serum except HDL-c. The mixture was then centrifuged for 5 minutes at 3000rpm. 0.1 ml of the supernatant was added to 5ml of absolute ethanol and allowed for 5 minutes before centrifuged. HDL-c was determined in the supernatant as described for cholesterol.

$$\text{HDL-c} = \frac{\text{Test} \times 5.2\text{mmol}}{\text{Standard}}$$

Determination of Triglycerides

In this method[18], three tubes labeled sample, blank, and standard were used. The sample and standard preparations were pipetted into the labeled test tubes. 0.01ml of distilled water was put into the blank test tube. The Sullivan reagent was added across the test tubes and incubated for 5 minutes at normal body temperature. The absorbance of the unknown and standard were measured against the blank at 500nm using the spectrophotometer. Triglyceride concentration was calculated as:

$$\text{Triglyceride (TG)} = \frac{\text{Absorbance of sample} \times \text{conc. of standard}}{\text{Absorbance of standard}}$$

Estimation of LDL-c

The equation of Friedewald *et al*., (1972) was used to estimate LDL-c level.

$$\text{LDL-c} = \text{Total cholesterol} - \text{HDL-c} + \frac{\text{TG}}{}$$

Estimation of total proteins (Without precipitations)
Principle

A cupric ion in alkaline solution react with the peptide bonds in proteins producing colour which is proportional to the amount of proteins present (Biuret reaction). The Technique uses Biuret working solution test at 5.9ml and standard at 5.9ml while the test for serum was 0.1 ml with the standard for serum at 0.1. It was Incubated and allowed to stand at room temperature for 10minutes in a water bath and read at 540nm against water blank.

Calculation: T/S x concentration of standard serum
T/S x 6g/100ml (60-80g/L).

Normal range: 6-8.0 g/100ml (60-80g/L).

Estimation of serum albumin
Principle

The dye – Bromocresolgreen has a specific affinity for albumin in acidic solution. Therefore the albumin – dye complex so formed can be estimated colorimetrically at 628nm wavelengths.[19]

It was mixed well and read against blank at 620nm

Calculation

Albumin level = T/S x Concentration of Std.
= T/S x 4g/100ml (40g/L)

Normal Values: 3-5g/100ml (30-50g/L)

Determination of serum total protein

The Bradford protein assay by colorimetric method was used. The principle of this assay is that the binding of protein molecules to coomassie dye under acidic conditions results in a colour change from brown to blue. This method actually measures the presence of the basic amino acid residues, arginine, lysine and histidine, which contributes to formation of the protein-dye complex. Five standard solutions (1 ml each) containing 0, 10, 20, 30, 40 and 50 µg m1-1 Assay was prepared according to the manufacturer's instruction in which 800 µl of each standard and sample solution (containing for <50 µg ml-1 protein) was dispensed into a clean, dry test tube and 200 µl of dye reagent concentrate were added to each tube and mixed samples were incubated at room temperature for at least 5 minutes but not more than 1 hour. Absorbance was measured and recorded at 595 nm.

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 Total protein

The mean serum total protein in control group, GL only, DM+GL, DM+Insulin, and DM only was 44.0 ± 0.436g/l, 41.6 ± 0.481g/l, 39.3 ± 0.471g/l, 48.8 ± 0.416g/l, and 50.0 ± 0.577g/l, respectively. The results present a significant ($p<0.05$) increase in total proteins in the DM group compared with the control. Treatment with GL reversed it towards normal. This is seen in figure 1.

Effect of GL on Serum Albumin

The mean serum albumin level in control group, GL only, DM+GL, DM+insulin, and DM only was 15.9±0.508g/dl, 17.7±1.70g/dl, 15.4±0.481g/dl, 20.7±0.421, and 18.7±0.843 respectively. The result shows a significant ($p<0.05$) increase in DM group compared with the control. Treatment with GL reversed it towards normal. This is presented in figure 2.

Effect of GL on Serum globulin

The mean serum globulin level in the control group, GL only, DM+GL, DM+Insulin, and DM only was 28.1±0.340g/dl, 23.3±1.15g/dl, 24.3±0.408g/dl, 28.5±0.401g/dl and 30.3±0.615g/dl respectively. Serum globulin was significantly ($p<0.05$) increased in DM group compared with the control group. Treatment with GL significantly ($p<0.01$) decreased it towards normal. This is presented in figure 3.

Effect of on Total cholesterol (TC) level

The mean serum TC level in the control, GL only, DM+GL, DM+Insulin, and DM only groups was 1.53±0.0421mmol/l, 2.33±0.245mmol/l, 3.01±0.0738mmol/l, 3.83±0.0286mmol/l. and 6.28±0.145mm/l, respectively. The result showed a significant ($p<0.05$) increase in TC in the DM group compared with control. Treatment with GL reduced TC towards normal. This is seen in figure 4.

Effect of GL on Serum high density lipoprotein-cholesterol levels (HDL-C)

The mean serum HDL-C level in the control, GL only, DM+GL, DM+Insulin, and DM only groups was 1.7±0.021mml/L, 1.6±0.042mml/L, 1.0±0.034ml/L, 0.85±0.017ml/L, and 0.40±0.012ml/L, respectively. The result shows a significant ($p<0.05$) decrease HDL-c in the DM group compared with the control. GL treatment increased the HDL-c towards normal. This is seen in figure 5.

Effect on GL on Serum low density lipoprotein-cholesterol levels (LDL-C)

The mean serum LDL level in the control, GL only, DM+GL, DM+Insulin, and DM only groups was 0.87±0.032mm/l, 1.7±0.16mm/l, 2.0±0.070mm/l, 2.5±0.031mm/l. and 4.5±0.10mm/l, respectively. The result shows a significant ($p<0.05$) increase level of LDL-c in the DM group compared with the control. Treatment with GL reversed it towards normal. This is seen in figure 6.

Effect of GL on Serum triglyceride concentration

The mean serum TG concentration in the control, GL only, DM+GL, DM+Insulin, and DM only groups was 0.56±0.010mmol/L, 0.66±0.0090mmol/L, 0.46±0.0068mmol/L, 0.64±0.0092mmol/L. and 1.7±0.022mmol/L, respectively. Serum TG concentration showed a significant ($p<0.05$) increase in the DM group compared with control. But treatment with GL reduced the TG concentration towards normal. This is presented in figure 7.

Effect of GL on Very low density lipoprotein cholesterol

The mean serum VLDL in the control, GL only, DM+GL, DM+Insulin, and DM only groups was 0.25±0.0036mmol/L, 0.30±0.0044mmol/L, 0.21±0.0057mmol/L, 0.29±0.0057mmol/L. and 0.76±0.019mmol/L, respectively. The result shows a significant ($p<0.05$) increased in the DM group compared with control. But treatment with GL reduced it towards normal. This is presented in figure 8

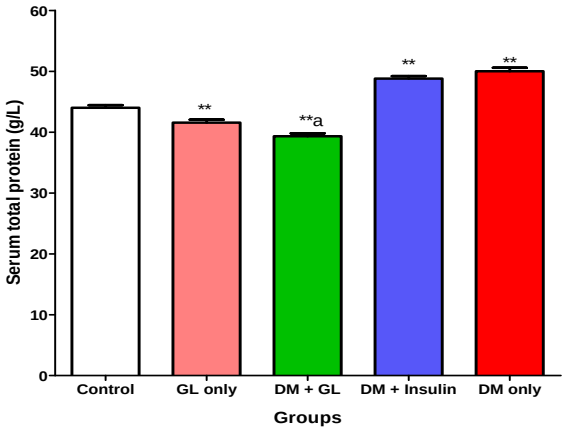

Fig.1: Comparison of Serum total protein level in the different experimental groups.
Values are expressed as mean ±SEM, n = 5.
* = p<0.05 compared with control;
** = p<0.01 compared with control;
a = p<0.05 compared with DM+ insulin

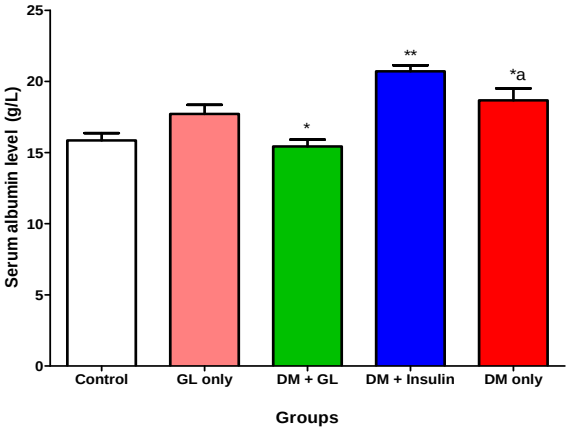

Fig.2: Comparison of Serum albumin level in the different experimental groups.
Values are expressed as mean ±SEM, n = 5.
*=p<05 versus GL only;
**=p<0.01 versus control,
a=p<0.05 compared with DM+insulin

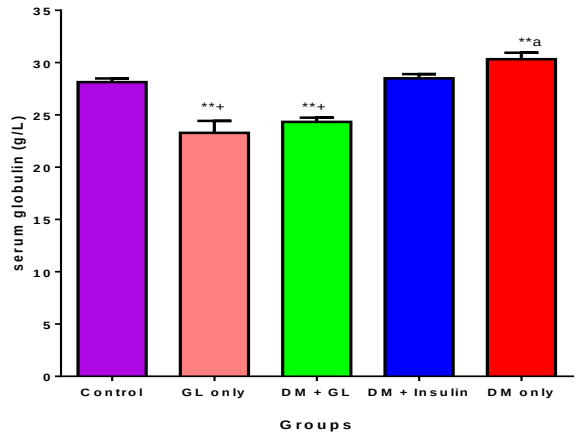

Fig. 3: Comparison of Serum globulin level in the different experimental groups.

Values are expressed as mean ±SEM, n = 5.
* = p<0.05 compared with control;
** = p<0.01 compared with control;
a = p<0.05 compared with DM+ insulin

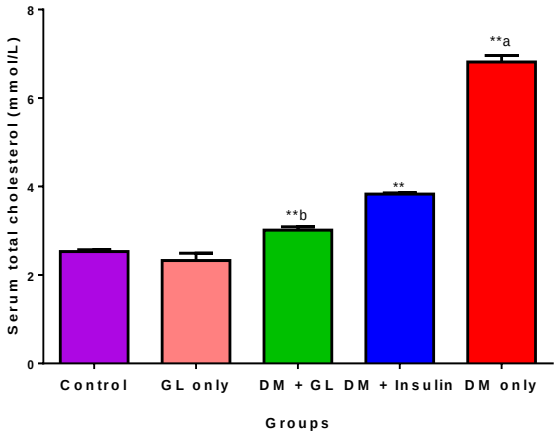

Fig. 4: Comparison of Serum total cholesterol level in the different experimental groups.

Values are expressed as mean ±SEM, n = 5.
** = p<0.01 compared with control;
a = p<0.01 versus DM+insulin;
b =p<0.05 compared with GL only group

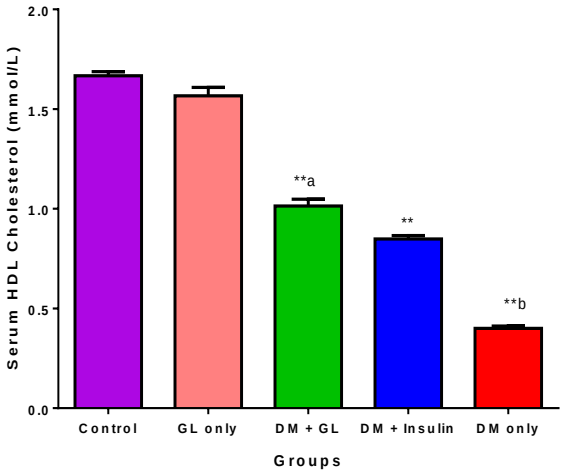

Fig.5: Comparison of Serum high density lipoprotein cholesterol level in the different experimental groups.

Values are expressed as mean ±SEM, n = 5.
**=p<0.01 compared with control;
a = p<0.01 compared with GL and DM+insulin;
b = P<0.01 compared with DM+insulin

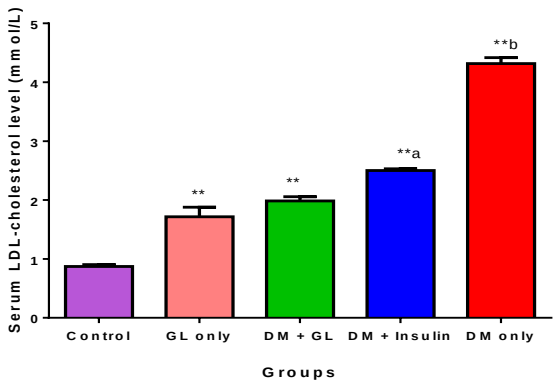

Fig. 6: Comparison of Serum low density lipoprotein cholesterol level in the different experimental groups.
Values are expressed as mean ±SEM, n = 5.
**=p<0.01 compared with control;
a =p<0.05 versus DM+GL and DM only group

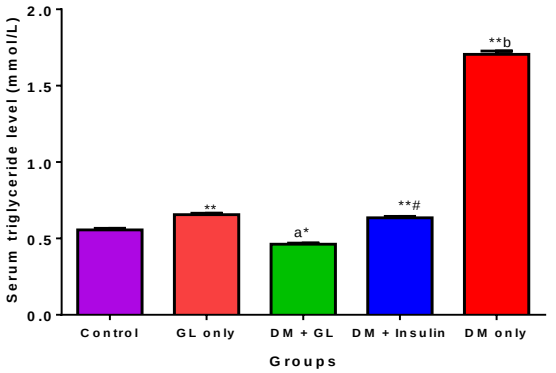

Fig.7: Comparison of Serum triglyceride level in the different experimental groups.

Values are expressed as mean ±SEM, n = 5.
**=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 group;
# = p<0.05 compared with DM+GL group

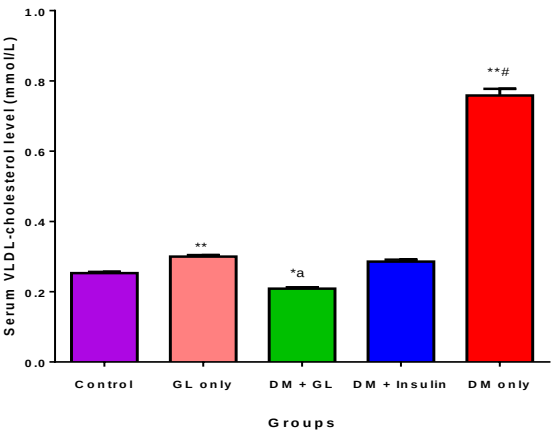

Fig.8: Comparison of Serum very low density lipoprotein cholesterol level in the different experimental groups.

Values are expressed as mean ±SEM, n = 5.
**=p<0.05 compared to all other groups,
a = p<0.05 compared with DM+ GL

DISCUSSION

This study was aimed at investigating the effect of ethanolic leaf extract of *Gongronema latifolium* on serum lipid profile and plasma protein levels 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.

Serum proteins are for repair of damage tissues during disease condition. A decrease in total serum protein in *Gongronema latifolium* treated group when compared with the insulin treated group showed an effective control of total protein which can be attributed to improvement in the hepatic cell secretory mechanism.[20] Also a significant decrease in globulin and albumin level in the GL+DM treated group shows that the integrity of the cell membrane in the liver was not compromised which provides evidence of liver protection by *Gongronema latifolium* due to the presence of the phytochemical flavonoid in the plant extract.

The liver has a central role in the maintenance of lipid homeostasis and the presence of toxicants may alter the concentration of serum lipids which could increase the risk of atherosclerosis since increased LDL cholesterol and decreased HDL cholesterol levels are risk factors of atherosclerosis and related cardiovascular diseases. The present results on lipid profile showed a significant decrease in serum levels of LDL-cholesterol, VLDL- and total cholesterol, triglyceride levels and a raised serum HDL-cholesterol level in DM rats treated with GL. The observed reduction in the serum lipid levels except HDL-cholesterol compared with insulin treated group indicates that *G. latifolium* leaves extract could have hypolipidemic properties. This is consistent with previous studies that reported hypolipidaemic property of *G. latifolium* extracts in animal model.[21,22]

CONCLUSION

It is therefore concluded that *Gongronema latifolium* leaf extract has hypolipdemic property and play a role in reducing the elevated plasma proteins during diabetic rats induced with STZ.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes care*, 2013; 36(1): S67.
2. Kumar S, Behl T, Sachdeva M, Sehgal A, Kumari S, Kumar A, Kaur G, Yadav HN, and Bungau S. Implicating the effect of ketogenic diet as a preventive measure to obesity and diabetes mellitus. *Life sciences*, 2021; 264: 118661.
3. Mahe 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.
4. Barron E, Bakhai C, Kar P, Weaver A, Bradley D, Ismail H, Knighton P, Holman N, Khunti K, Sattar N, and Wareham NJ. Associations of type 1 and type 2 diabetes with COVID-19-related mortality in England: a whole-population study. The lancet Diabetes & endocrinology, 2020; 8(10): 813-822.
5. Brown AM, Rich LR, Ransom BR. Metabolism of Glycogen in Brain White Matter. *Brain Glycogen Metabolism*, 2019; 13: 187-207.
6. 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.
7. 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.
8. Rashidat T, Ibitayo, A. Growth response, nutrient utilization, biochemical and hematological parameters of Juvenile African catfish (clarias gariepinus) fed diet supplemented with gongronema latifolia (benth) extract. *Egyptian Journal of Aquatic Biology and Fisheries*, 2018; 22(4): 67-179.
9. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *Journal of biosciences*, 2003; 28(1): 1-5.
10. Morebise O, Fafunso MA, Makinde JM, Olajide OA, Awe EO. Antiinflammatory property of the leaves of Gongronema latifolium. *Phytotherapy Research*, 2002; 16(S1): 75-77.
11. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *Journal of biosciences*, 2003; 28(1): 1-5.
12. Ugochukwu NH, Cobourne MK. Modification of renal oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with extracts from Gongronema latifolium leaves. *Clinica Chimica Acta*, 2003; 336(1-2): 73-81.
13. Wolkoff AW, Cohen DE. Hepatocyte transport of bile acids. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 2003; 284(2): G175-G179.
14. Ferrell JM, Chiang JY. Understanding bile acid signaling in diabetes: from pathophysiology to therapeutic targets. *Diabetes & metabolism journal*, 2019; 43(3): 257-272.
15. Fickert P, Stöger U, Fuchsbichler A, Moustafa T, Marschall HU, Weiglein AH, Tsybrovskyy O, Jaeschke H, Zatloukal K, Denk H, and Trauner M. A new xenobiotic-induced mouse model of sclerosing cholangitis and biliary fibrosis. *The American journal of Pathology*, 2007; 171(2): 525-36.
16. Gérard P, Lepercq P, Leclerc M, Gavini F, Raibaud P, Juste C. Bacteroides sp. strain D8, the first cholesterol-reducing bacterium isolated from human feces. Applied and environmental microbiology, 2007; 73(18): 5742-5749.15.
17. Agarwal H., Nakara, A., and Shanmugam, V. K. Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: A review. Biomedicine & Pharmacotherapy, 2019; 109: 2561-2572.
18. Siedel J, Hägele EO, Ziegenhorn J, Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clinical Chemistry*, 2, 1983; 9(6): 1075-1080.
19. Kazumi T, Yoshino G, Matsuba K, Iwai M, Iwatani I, Kasama T, Yoshida M, and Baba S. Bezafibrate lowers VLDL concentrations without modifying its structure. Hormone and metabolic research, 1989; 21(06): 341-2.
20. Yano Y, Grigoryan H, Schiffman C, Edmands W, Petrick L, Hall K, Whitehead T, Metayer C, Dudoit S, and Rappaport S. Untargeted adductomics of Cys34 modifications to human serum albumin in newborn dried blood spots. *Analytical & Bioanalytical Chemistry*, 2019; 411(11): 2351-62.
21. Rajkapoor B, Venugopal Y, Anbu J, Harikrishnan N, Gobinath M, Ravichandran V. Protective effect of Phyllanthus polyphyllus on acetaminophen induced hepatotoxicity in rats. Pak J Pharm Sci., 2008; 21(1): 57-62.
22. Okon IA, Ufot UF, Onoyeraye UG, Nwachukwu EO, Owu DU. Effect of Gongronema latifolium on lipid profile, oral glucose tolerance test and some hematological parameters in fructoseinduced hyperglycemia in rats. *Pharmaceutical and Biomedical Research*, 2019; 5(1): 25-31.