

Anti-inflammatory potentials of ethanolic leaf extract of *Gongronema latifolium* in streptozotocin induced diabetic rats

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Abstract

Introduction: Inflammation due to diabetes mellitus has been understudied. *Gongronema latifolium* (GL) is reported to have anti-inflammatory potentials. This work was designed to evaluate the effect of ethanolic leaf extract of *Gongronema latifolium* on some inflammatory markers in Streptozotocin (STZ)-induced diabetic rats.

Materials and methods: 40 Wistar rats of both sexes (150 g-200 g) were divided into 5 groups of eight (8) rats each. Group 1; control received normal saline placebo orally. Group 2; GL only received 200 mg/kg of GL orally. Group 3 DM only received 65 mg/kg bw of STZ intraperitoneally for two days with an interval of one day in between. Group 4; DM + GL received 65 mg/kg bw of STZ + 200mg/kg bw of GL. Group 5; DM + Insulin received 65mg/kgbw of STZ followed by 10 IU/kgbw of insulin subcutaneously. For further inflammatory study, paw oedema was induced by sub-plantar injection of 2% formalin in two groups before and after administration of 200 mg/kg bw of GL daily for 7 days. Blood samples were collected via cardiac puncture for biochemical analysis.

Results: Results from this study showed a significant ($p < 0.05$) increase in blood glucose DM group, compared to the control. Intervention with GL and insulin significantly reduced the glucose level towards normal. Interleukin-6 concentration and C-reactive proteins were significantly ($p < 0.01$) higher in DM group compared with the GL and control groups. Paw oedema size was significantly ($p < 0.05$) increased in the formalin treated group compared with the control. Pretreatment and post treatment with GL significantly ($p < 0.05$) reduced the paw oedema size.

Conclusion: In conclusion, GL (like insulin) has hypoglycemic and anti-inflammatory potentials.

Keywords: *Gongronema latifolium*; Diabetes; C-reactive peptide; Interleukin-6; Paw oedema

1. Introduction

Diabetes mellitus (DM) is a pathological and metabolic condition characterized by impaired glucose metabolism caused by inadequate insulin action or insulin resistance [1]. Clinically, it is defined as a fasting plasma glucose level >7.8 mmol/l (140 mg/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 (80-100 mg/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 [2]. It is a complicated and chronic disease with complex etiologies [3] which can lead to reduced glucose tolerance, nerve damage, kidney failure, atherosclerosis, stroke, blindness and heart disease.

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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 [4].

The early symptoms of diabetes include elevated blood sugar (glucosuria), dehydration, weight loss, increased food intake (polyphagia), increased water intake (polydipsia), increased urinary output (polyuria), and blurred vision. 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 [5].

The blood glucose concentration is controlled by a feedback system between liver, muscle, fat, and pancreatic islet cells and the overall pattern of control differing in basal and fed states. In Type 1 (insulin dependent diabetes mellitus), insulin is the main form of treatment, while in Type 2 (non-insulin dependent diabetes mellitus), diet is the cornerstone, often combined with oral hypoglycemic agent. Dietary management is essential in the management of both types of diabetes [6].

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 [7]. 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 [8].

The plant also has anti-inflammatory property and exhibits antimicrobial activities against various microbial pathogens [9].

Inflammation is a physiological response caused by an underlined medical condition, which result in increased production of white blood cells and other substances that protect the body against foreign pathogens such as bacteria and viruses [10]. It is the means the body signals the immune system to heal, repair damaged tissues and defend itself against foreign invaders. In disease conditions, the immune system triggers an inflammatory response when there are no foreign invaders to fight off [11]. However, prolonged inflammatory process can become problematic. Chronic inflammation has been linked to autoimmune disorders such as lupus and rheumatoid arthritis and may also lead to cardiovascular diseases such as heart attack or stroke [12].

Inflammatory biomarkers are also called Cytokines; these are the hormone-like small proteins acting as intercellular messengers by binding to specific receptors of target cells. These non-antibody proteins are produced by WBCs and some other types of cells. The major function of cytokines is the activation and regulation of general immune system of the body. Depending upon the source of secretion and effects, cytokines are classified into several types; Interleukins, interferons, tumor necrosis factors, chemokines, defensins, cathelicidins, platelet-activating factor.

Interleukins (IL) are the polypeptide cytokines which are produced mainly by the leukocytes. pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL-6 gene. In addition, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-1 α and IL-10 [13].

IL-6 is an important mediator of fever and of the acute phase response. It is capable of crossing the blood-brain barrier [13] and initiating synthesis of PGE₂ in the hypothalamus, thereby changing the body's temperature set point

C-reactive protein (CRP) is an annular, pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q [14].

CRP is synthesized by the liver [15] in response to factors released by macrophages and adipocytes [16]. C-reactive protein was the first pattern recognition receptor (PRR) to be identified [17].

There is paucity in scientific literature on the effect of GL on inflammatory makers, interleukin-6 and C-reactive proteins in diabetic condition. This study was therefore designed to elucidate the effect of GL on interleukin-6 and C-reactive protein levels in diabetic rats.

2. Material and methods

2.1. Preparation of *Gongronema latifolium* extract

The preparation of extract was according to standard method [18]. *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 70% ethanol (BDH Ltd Poole, England) in the evening (6:00 pm), 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.

2.2. Experimental animal

Ethical approval was obtained from the Faculty of Basic Medical Sciences University of Calabar Animal Research and Ethical Committee with ethical number: (019PY20317). 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 of 27±2 °C, with 12hours light-dark cycle.

2.3. Experimental design

The Wistar rats were divided into five groups (n=8). Group 1: Control, Group 2: Diabetic (DM), Group 3: *Gongronema latifolium* (GL), Group 4: DM+GL, Group 5: DM + Insulin

2.4. 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 the method of Ugochukwu[18]. Treatment lasted for 28 days.

2.5. 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).

2.6. 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 chloroform vapour and blood samples collected via cardiac puncture using sterile needles into plain and EDTA sample bottles. The blood samples in plain tubes were then centrifuged at 1000rpm for 10 minutes, serum collected and stored for subsequent biochemical analysis of inflammatory biomarkers.

2.7. Determination of Inflammatory Biomarkers

Interleukin-6 (IL-6) and C-reactive protein were measured by enzyme linked immunosorbent assay ELISA kits (Calbiotech Inc. and Elab Science, USA) according to manufacturer's instructions.

2.8. Induction of inflammation using formalin

In another set of experiment to test the anti-inflammatory property of the *Gongronema latifolium* leaf extract, paw oedema was induced with 2% formalin following standard method [19]. Oedema size was measured using Vernier caliper.

2.9. Data Analysis

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

3. Results

3.1. Effect of GL on Blood glucose level

The result for blood glucose level is presented in figure 1. The mean blood glucose values in the control, GL only group, DM + GL group, DM + Insulin group and DM group were 120 ± 5.5 mg/dl, 113 ± 6.3 mg/dl, 422.0 ± 3.4 mg/dl, 380 ± 25.0 , and 538 ± 14.0 mg/dl respectively. The result showed a significant ($p < 0.01$) decrease in the mean blood glucose levels in the GL treated groups and insulin group when compared with the DM and control group.

3.2. Effect of GL on Serum interleukin-6

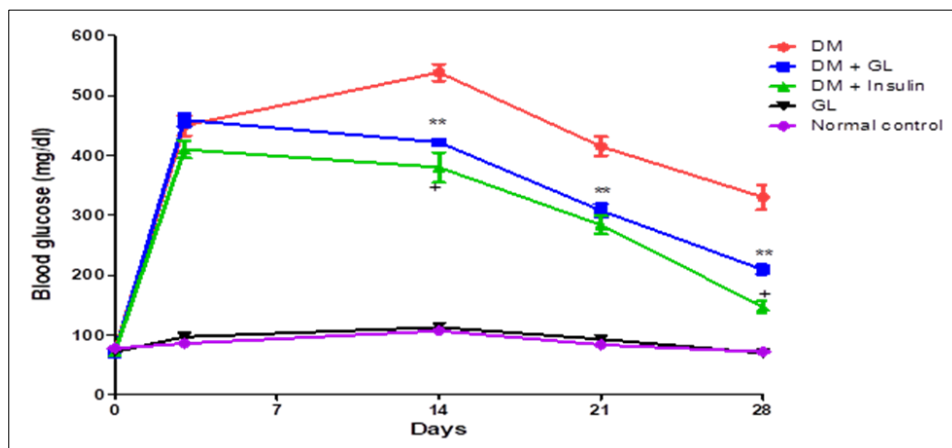
The result for serum interleukin-6 is presented in figure 2. The mean values of interleukin-6 in the control, GL only, DM+GL, DM+insulin, and DM group were 3.40 ± 0.118 pg/ml, 4.41 ± 0.112 Pg/ml, 5.39 ± 0.118 Pg/ml, 4.69 ± 0.099 pg/ml, and 9.32 ± 0.277 pg/ml respectively. The result showed a significant ($p < 0.005$) increase in serum interleukin-6 levels in the DM group compared with the control. Treatment with GL and insulin significantly reduced IL-6 towards normal.

3.3. Effect of GL on C-reactive protein

The result for C-reactive protein is presented in figure 3. The mean C-reactive protein values in the control, GL only, DM+GL, DM+insulin, and DM group were 2.27 ± 0.0585 ng/ml, 2.67 ± 0.0880 ng/ml, 3.26 ± 0.0481 ng/ml, 2.60 ± 0.0378 ng/ml and 5.20 ± 0.0931 ng/ml respectively. The result presents a significant ($p < 0.05$) increase in C-reactive protein in the DM group when compared with the control. This effect was attenuated with GL and insulin treatments.

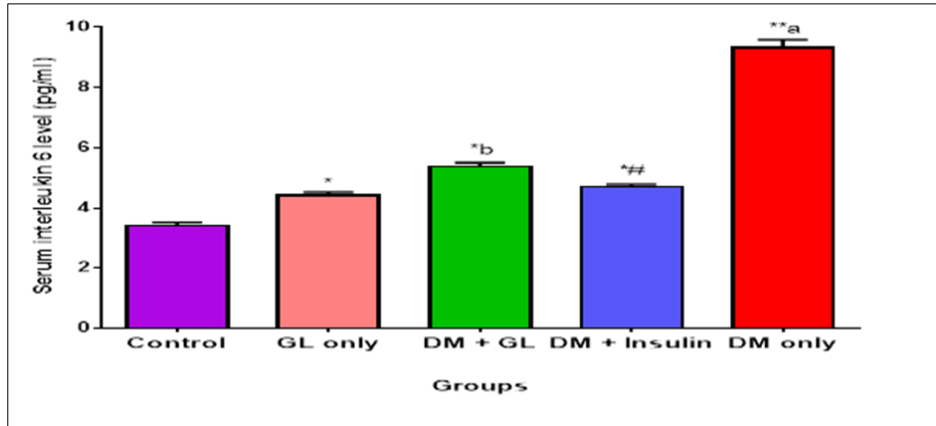
3.4. Effect of GL on Paw oedema size

The result to confirm paw edema size is presented in figure 4. The mean oedema size of the formalin control group, GL before formalin group, and formalin before GL group was 11 ± 0.3 mm, 7.2 ± 0.1 mm and 8.6 ± 0.2 mm respectively. The result showed a significant ($p < 0.05$) decrease in paw oedema size in the pre and post GL treated groups compared with the formalin control group.



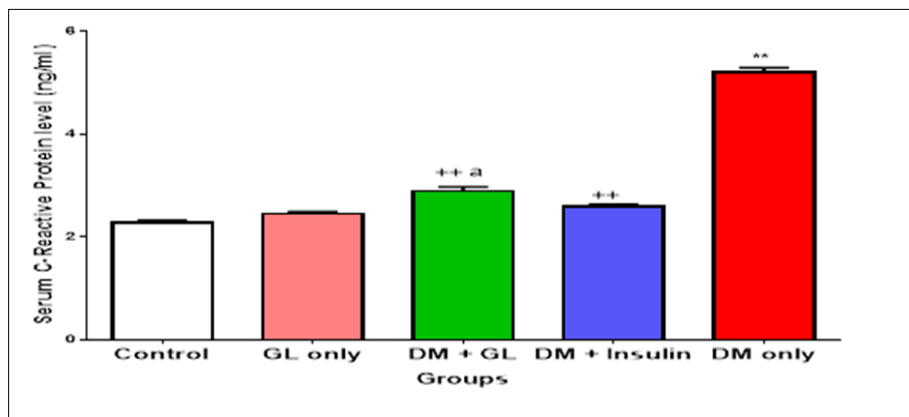
** = $p < 0.01$ compared with DM group; + = $p < 0.05$ compared with DM group

Figure 1 Effect of GL on mean blood glucose level



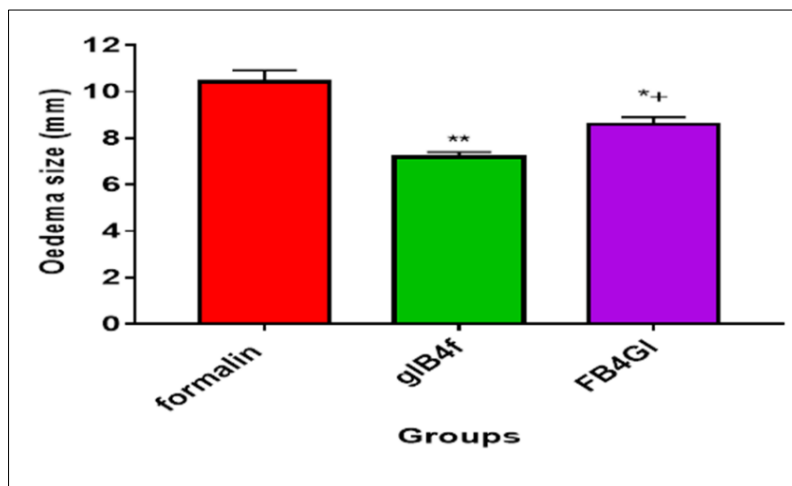
* = p<0.05 compared with control; ** = p<0.01 compared with control; a = p<0.05 compared with DM+ insulin; b = p<0.05 compared with GL only

Figure 2 Effect of GL on serum interleukin-6



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

Figure 3 Effect of GL on Serum C-reactive protein



** = p<0.01 compared with formalin; * = p<0.05 compared with GLb4f; + = p<0.05 compared with formalin group

Figure 4 Effect of GL on Paw oedema size

4. Discussion

This study was aimed at investigating the effect of ethanolic leaf extract of *Gongronema latifolium* on some inflammatory biomarkers in streptozotocin-induced diabetic rats. And the effect of the extract on blood glucose levels of rats treated with *G. latifolium* and insulin were also determined.

The decrease in blood glucose levels seen in animals treated with insulin and GL is in agreement with previous studies that reported hypoglycaemic effect of GL in diabetic condition [8, 20]. *Gongronema latifolium* contain phytochemicals with a combine potential for reducing blood glucose level. Phytochemicals present in *Gongronema latifolium* include flavonoid, polyphenols, coumarins, saponins, tanins and alkaloids. Studies have shown that phytochemicals like flavonoid and glycosides have hypoglycaemic effect by inhibiting alpha glucosidase enzyme or by regeneration of the damaged pancreatic beta cells [21]. *G. latifolium* can ameliorate this damage due to its anti-diabetic and anti-inflammatory properties.

Inflammation is a secondary response by a tissue due to an injured surrounding tissue. It is the body's immune system's response to an irritant [22]. During DM, there is increased production of inflammatory markers from fat tissues. This abnormal inflammation alters insulin's action and contributes to the disease [23]. The result showed a beneficial decrease in serum interleukin- 6 and C-reactive protein levels in diabetic group treated with *Gongronema latifolium* and insulin groups. Oxidative stress due to increase production of free radicals in diabetic condition can also increase levels of inflammatory markers or cytokines [24].

The result of this study shows that *Gongronema latifolium* has anti-inflammatory and anti-diabetic properties. The flavonoids exhibits anti-inflammatory role by the inhibition of the synthesis and activities of different pro-inflammatory mediators such as C-reactive protein, interleukin-6 cytokines and adhesion molecules [25]. Molecular activities of flavonoids include inhibition of transcription factors such as NF-kappa B and activating protein-1 (AP-1), as well as activation of nuclear factor-erythroid 2-related factor 2 (Nrf2). These properties help to ameliorate the effect of inflammation and oxidative damage caused by DM.

C- reactive protein is an acute-phase protein with several advantages over other acute-phase reactants, this is due to its pronounced rise in concentration after tissue injury or inflammation [26]. It is a highly sensitive marker of systemic (micro)-inflammation, tissue damage and infection [27]. Elevated level of CRP and IL-6 predict the development of inflammation in DM. Inflammation of the blood vessel wall is considered to play an essential role in the initiation and progression of atherosclerosis [28]. The result presented above showed a decrease in C-reactive protein and interleukin-6 level in *Gongronema latifolium* and insulin treated group when compared with diabetic (DM only) group. The decrease in the C-reactive protein levels in the two treated group is an indication of the anti-inflammatory potential of *G. latifolium* which may be due to the flavonoid and polyphenols in the plant extract [29]. A study reported that flavonoids present in *G. latifolium* leaves extract are kaempferol, kaempferol 3-O-rutinoside, rutin and laricitrin-3- O-glucoside [30]. Kaempferol and the rutinoside, and rutin have anti-inflammatory and antioxidant activity [31, 32]. Pre and post administration of GI reduces paw-oedema size due to the injection of formalin which point to the fact that GI has both preventive and curative potentials in paw-oedema.

5. Conclusion

In conclusion, *Gongronema latifolium* has anti-inflammatory and antidiabetic properties evidenced by the reduction of inflammatory biomarkers, and blood glucose. The anti-inflammatory, antidiabetic properties of *Gongronema latifolium* may be due to the presence of antioxidants such as flavonoids saponins and tannins which protect the cells against oxidative damage. Thus, *Gongronema latifolium* use as spice in our diet may help in reducing the risk of inflammation and oxidative stress damage caused by diabetes mellitus.

Compliance with ethical standards

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Author Contributions

Concept, design, and supervision by D.U.O, J.A.B.; Resources, materials, data collection and processing by G.O.U, I.A.O; Analysis and interpretation: D.U.O. ; Literature search and manuscript writing: G.O.U; Critical review: J.A.B.

Disclosure of conflict of interest:

None of the authors have a conflict of interest

Statement of 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).

Statement of informed consent

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

References

- [1] 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 sciences*. 2021; 264: 118661.
- [2] 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 Diabetes & endocrinology*. 2020; 8(10): 813-822.
- [3] Riddle MC, Philipson LH, Rich SS, Carlsson A, Franks PW, Greeley SAW, Nolan JJ, Pearson ER, Zeitler PS, Hattersley AT. Monogenic diabetes: from genetic insights to population-based precision in care. Reflections from a diabetes care editors' expert forum. *Diabetes Care*. 2020; 43(12): 3117-3128.
- [4] 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.
- [5] 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.
- [6] Cogen FR. Incorporation of the Ketogenic Diet in a Youth with Type 1 Diabetes. *Clinical Diabetes*. 2020; 38(4): 412-415.
- [7] 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 fructose induced hyperglycemia in rats. *Pharmaceutical and Biomedical Research*. 2019; 5(1): 25-31.
- [8] Owu DU, Nwokocha CR, Obembe AO, Essien AD, Ikpi DE, Osim EE. Effect of Gongronema latifolium ethanol leaf extract on gastric acid secretion and cytoprotection in Streptozotocin-induced diabetic rats. *West Indian Medical Journal*. 2012; 61(9): 112- 119
- [9] Antai AB, Ofem OE, Ikpi DE, Ukafia S, Agiang E. A phytochemistry and some haematological changes following oral administration of ethanolic root extract of Gongronema latifolium in rats. *Nig J Physiol Sci*. 2009; 24(1): 79–8.
- [10] Karimy JK, Reeves BC, Damisah E, Duy PQ, Antwi P, David W, Wang K, Schiff SJ, Limbrick DD, Alper SL, Warf BC. Inflammation in acquired hydrocephalus: pathogenic mechanisms and therapeutic targets. *Nature Reviews Neurology*. 2020; 16(5): 285-296.
- [11] Duris K, Jurajda M. Evolutionary concept of inflammatory response and stroke. *J Neurosci Res*. 2020; 98(1): 98-104.
- [12] Mohler A, Montalvo M, Roth J. Paraneoplastic and Other Autoimmune Disorders. In *Clinical Neuroimmunology*. Humana, Cham. 2020; 235-255.
- [13] Banks WA, Kastin AJ, Gutierrez EG. Penetration of interleukin-6 across the murine blood-brain barrier. *Neurosci Letters*. 1994; 179(1–2): 53–6.

- [14] Mishra AK, Kumar S, Kumar P, Choudhary MK, Kumar S, Ahmad A, Kumar N, Pandey J. Level of C-reactive Protein in Obese and Overweight Individuals at Indira Gandhi Institute of Medical Sciences: A Tertiary Care Center of Bihar. *Inter J Scientific Study*. 2020; 8(3): 110-112.
- [15] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *The J Clin Investigation*. 2003; 111(12): 1805–1812.
- [16] Wen J, Liang Y, Wang F, et al. C-reactive protein, gamma-glutamyltransferase and type 2 diabetes in a Chinese population. *Clin Chim Acta*. 2010; 411(3–4): 198–203.
- [17] Mustafa ES, AL-Tae SK. Innate and adaptive immunity in fish: a review. *Al-Anbar J Vet Sci*. 2020; 13: 1-16.
- [18] Ugochukwu NH, Babady NE. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life sciences*. 2003; 73(15): 1925-1938.
- [19] Kumar T, Jain V. Antinociceptive and anti-inflammatory activities of *Bridelia retusa* methanolic fruit extract in experimental animals. *The Scientific World Journal*. 2014; 1-12.
- [20] Adeeyo AO, Odelade KA, Msagati TA, Odiyo JO. Antimicrobial potencies of selected native African herbs against water microbes. *Journal of King Saud University-Science*. 2020; 32(4): 2349-2357.
- [21] Kanu AN, Ezeocha CV, Ogunka NP. A review on bioactive compounds of yam varieties for human disease management. *Asian Food Sci. J*. 2018; 1(4): 1-10.
- [22] Babensee JE. Inflammation, Wound Healing, the Foreign-Body Response, and Alternative Tissue Responses. *In Biomaterials Science*. 2020; 737-746.
- [23] Hartmann K, Koenen M, Schauer S, Wittig-Blaich S, Ahmad M, Baschant U, Tuckermann JP. Molecular actions of glucocorticoids in cartilage and bone during health, disease, and steroid therapy. *Physiological reviews*. 2016; 96(2): 409-447.
- [24] Choromańska B, Myśliwiec P, Dadan J, Maleckas A, Zalewska A, Maciejczyk M. Effects of age and gender on the redox homeostasis of morbidly obese people. *Free Radical Biology and Medicine*. 2021; 175: 108-120.
- [25] Hughes SD, Ketheesan N, Haleagrahara N. The therapeutic potential of plant flavonoids on rheumatoid arthritis. *Critical Reviews FoodSci Nutri*. 2017; 57(17): 3601-3613.
- [26] Pepys MB. The acute phase response and C-reactive protein. *Oxford Textbook of Medicine*. 1995; 2: 1527-533.
- [27] Ridker PM. C-reactive protein, inflammation, and cardiovascular disease: clinical update. *Texas Heart Institute Journal*. 2005; 32(3): 384.
- [28] Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Research & Therapy*. 2006; 8(2): S3.
- [29] Mihailović M, Dinić S, Arambašić Jovanović J, Uskoković A, Grdović N, Vidaković M. The influence of plant extracts and phytoconstituents on antioxidant enzymes activity and gene expression in the prevention and treatment of impaired glucose homeostasis and diabetes complications. *Antioxidants*. 2021; 10(3): 480.
- [30] Beshel JA, Palacios J, Beshel FN, Nku CO, Owu DU, Nwokocha M, Bórquez J, Simirgiotis MJ, Nwokocha CR. Blood pressure-reducing activity of *Gongronema latifolium* Benth. (Apocynaceae) and the identification of its main phytochemicals by UHPLC Q-Orbitrap mass spectrometry. *J Basic Clinical Physiol Pharm*. 2020; 31(1): 234-239
- [31] Wang J, Fang X, Ge L, Cao F, Zhao L, Wang Z, Xiao W. Antitumor, antioxidant and anti-inflammatory activities of kaempferol and its corresponding glycosides and the enzymatic preparation of kaempferol. *PLoS One*. 2018; 13(5): e0197563.
- [32] da Silva AB, Cerqueira Coelho PL, das Neves Oliveira M, Oliveira JL, Oliveira Amparo JA, da Silva KC, Soares JRP, Pitanga BPS, Dos Santos Souza C, de Faria Lopes GP, da Silva VDA, de Fátima Dias Costa M, Junier MP, Chneiweiss H, Moura-Neto V, Costa SL. The flavonoid rutin and its aglycone quercetin modulate the microglia inflammatory profile improving antiangioma activity. *Brain Behaviour Immunity*. 2020; 85: 170-185.