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.
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-1α 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 lyso phosphatidylcholine 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 plane and EDTA sample bottles. The blood samples in plane 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 ± 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
Figure 2 Effect of GL on serum interleukin-6

Figure 3 Effect of GL on Serum C-reactive protein

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 larintrin-3-O-gluco side [30]. Kaempferol and the rutinoside, and rutin have anti-inflammatory and antioxidant activity [31, 32]. Pre and post administration of GL reduces paw-oedema size due to the injection of formalin which point to the fact that GL 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.

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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.

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