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Original Research Article

Gongronema latifolium Ethanol Crude Leaf Extract Ameliorates Organ Injuries Via Anti-Inflammatory and Anti-Diabetic Mechanisms in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

In Streptozotocin-induced diabetes mellitus rats, the effects of *Gongronema latifolium (GL)*leaf extract on the histology of some organs, hyperglycemia, and inflammatory markers were investigated. Five (5) groups of 25 male rats (weighing 150g and 200g each) were created and group one received distilled water. Group 2 received GL extract at a dose of 200 mg/kg. Group 3 diabetic rats were only given access to water and food. Diabetes-induced rats in Group 4 received 200 mg/kg of GL extract. In group 5 rats, subcutaneous insulin (0.14 ml/kg) was given to the diabetic rats. The DM-induced group (t=3.892) had statistically higher plasma glucose levels (p=0.018) than the control group. In comparison to the diabetic non-treated group, the treatment groups receiving insulin (t=2.825, P=0.048) and extract (t=2.731, P=0.052) demonstrated a statistically significant decrease in plasma glucose level. The test groups showed lower serum levels of C-reactive protein (F=0.680, P=0.863), interleukin-6 (F=0.484, P=0.392), and MDA (F=0.347, P=0.108). Interleukin-6 and C-reactive protein levels in *Gongronema latifolium* leaf extract were reduced, demonstrating the plant's anti-inflammatory capabilities. Compared to the control group, the treated groups visceral organ histomorphology displayed characteristics compatible with normal histology of the organs. The group with diabetes mellitus-induced had glomeruli with focal regions of haemorrhage In the pancreas tissue of the diabetic non-treated group, cellular degradation was seen, indicating damage. Untreated diabetics exhibited a clogged central vein and an invasion of inflammatory cells. According to this study, *Gongronema latifolium* has anti-hyperglycemia and anti-inflammatory capabilities and reduces cellular changes in the visceral organs.

Keywords: *Gongronema latifolium*, Diabetes mellitus, C-reactive protein, interleukin-6, Malondialdehyde

Introduction

The rainforest plant called *Gongronema latifolium* is utilized in traditional medicine as a spice and vegetable salad. It is a member of the Kingdom of plants, *Assclepidaceae*as family, *Magnoliphyta* as a Phylum, *Magnoliopsida* as a Class, *Gentianales* as an Order, *Gongronema latifolium* as a Genus, and *Gongronema latifolium* as a Species.[1] In South Eastern and South Western Nigeria, it is referred to as "Utazi and Arokeke" frequently.[2]

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The climbing shrub *Gongronema latifolium* has woody, hollow stems, and roots. It has a delicate stem and heart-shaped, broad, and oval leaves. Flowering *Gongronema latifolium* is a seasonal plant. It is frequently observed in Nigeria throughout the rainy season. Laxative usage is just one of the many medical uses for G*ongronema latifolium*.[3,4] It is a wonderful source of vitamins and minerals and has a high protein content of about 27.2%.[3,4]. Its ethanolic extract contained alkaloids, saponins, tannins, cardiac glycosides, flavonoids, and polyphenols among other phytochemical components.[5]

A metabolic ailment called diabetes mellitus is frequently linked to a high disease burden in underdeveloped nations like Nigeria.[6,7] Due to the National Health Insurance Scheme's poor performance, the cost of diabetes care is relatively high.[8] Pain, swelling, heat, and loss of function are the hallmarks of the inflammatory response to injury.[9] In reaction to injury, endothelial cells create pro-inflammatory cytokines, which cause an increase in blood flow as well as fluid and protein movement.[10] Several physical, chemical, and vascular factors as well as the immune system in response to damage or infection induce inflammation. Chronic inflammation is sparked by resistance and is linked to problems from diabetes mellitus.[11] It has been suggested that this ongoing inflammatory response contributes to human cancer, as well as systemic diseases and organ damage. To treat metabolic

problems and antioxidant attacks, medicinal herbs that can reduce chronic inflammatory response are sought after.[12] The objective of the study was to assess how *Gongronema latifolium* affected hyperglycemia, inflammatory and antioxidant indicators, and the histomorphology of specific visceral organs of the animals involved.

Materials and Methods

Collection and preparation of *Gongronema latifolium* leaf extract
In Akamkpa forest in Cross River State, Nigeria, the leaves of the *Gongronema latifolium* were gathered in January 2017. Mr. EffaEffa of the Plant and Ecological Studies Department Herbarium, University of Calabar, Calabar, Nigeria, correctly identified and verified the plant. Herb/Bot/UCC/0719 was the voucher number allotted to it. After being cleaned and dried, the leaves were ground up with an electric blender. Following 72-hour maceration in 1250 ml of ethanol (BDH Ltd. Poole, England), 400g of the powdered leaves were filtered through Whatman No. 1 filter paper and then cellulose filter paper. Using a vacuum rotatory evaporator (Caframo, VV2000, Ohio), the filtrate was dried at 30^0C. The yield from the extraction was 172 g or 4.3% of the total weigh.
To acquire the dosage concentration, the ethanol extract was reconstituted to a stock of 500 mg/ml in 0.9% saline. The Rats were orally given a dosage of 200mg/kg via an orogastric tube.

Experimental animals
Twenty-five animals were employed, and they were kept in plastic cages at a temperature of 28 to 30^{0C} with a 12-hour cycle of light and darkness. Before the experiment began, they were acclimated for two weeks while receiving meals and water as needed. A single intraperitoneal injection of freshly produced Streptozotocin (STZ, Sigma, St. Louis, MO, USA) in citrate buffer (0.1 M, pH 4.5) at the dose of 65 mg/kg was used to induce diabetes after an overnight fast. Through a tail puncture, a fasting blood sugar concentration of (200mg/dl) was used to diagnose diabetes mellitus. This was done 72 hours after induction for groups 3, 4, and 5 using a glucometer and strips (Accu-Check, Roche, Germany). The work was completed following the Ethical approval by the Faculty of Basic Medical Sciences, University of Calabar Nigeria's Ethical Committee on Animal Use. The approval number FAREC/PA/UC/049.
Experimental design
Twenty-five Sprague Dawley Rats aged 2-3 months weighing 150-200g were used as follows;
Group 1-Normal rats were administered distilled water.
Group 2- Normal rats were administered 200mg/kg of *Gongronema latifolium* extract.
Group 3- Diabetic rats with no treatment.
Group 4- Diabetic rats were administered 200mg/kg of *Gongronema latifolium* extract.
Group5 - Diabetic rats were administered 0.14ml/kg of insulin subcutaneously.

Collection of tissues for biochemical assay and histology
After 28days of treatment with extract and standard drug insulin, the animals fasted for 12hours overnight, and fasting blood glucose levels were determined using Accu-Check Glucometer. On the 29th day, the rats were sacrificed under halothane anesthesia. Blood samples were collected through cardiac puncture. Liver, kidney, and pancreas tissues were excised for histological processing.

Determination of interleukin-6
Interleukin-6 (IL-6) was estimated using IL-6 enzyme-linked immunosorbent assay (ELISA) kit manufactured by KrishgenBiosystems, Mumbai. The IL-6 was assayed using ELISA and a reader as described by Nnodi *et al*[14] *and Abert etal*[15] The quantitative sandwich enzyme immunoassay method was used in this assay. A microplate had an anti-IL-6 antibody pre-coated on it. Any IL-6 that was present bound by the immobilized antibody and washed after standards and test samples were pipetted into the wells. The wells included an IL-6-specific biotin-conjugated antibody. Avidin-conjugated horseradish peroxidase (HRP) was applied and then washed after washing. The amount of IL-6 bound in the first stage was proportionate to the amount of substrate solution supplied. The color development was stopped and the intensity of the color was measured using a spectrophotometer microplate reader.

Determination of malondialdehyde (MDA)
Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substance formation according to the methods of Ohkawa *et al.*[16], and Ohishi *et al*[17] · 1.6 mL of TrisKCl buffer and 0.5 mL of 30% trichloroacetic acid (TCA) were added to an aliquot of 0.4 mL supernatant. After that, 0.5mL of 0.75% TBA was added, and the mixture was heated in a water bath for an hour. This was centrifuged at 3000g after being chilled in ice. The absorbance of the clear supernatant was measured at 532 nm in comparison to a reference blank of distilled water by the method of Todorova *et al*[18] and expressed as nmol of MDA/g of wet tissue using a molar extinction coefficient of the chromophore (1.56×10^{-5}-/m/cm).

Determination of C-reactive protein
The quantitative sandwich enzyme immunoassay technique was used. On a microplate, CRP-specific antibody was previously coated. Standards and samples were pipetted into the wells, and the immobilized antibody bound any CRP that was present. A biotin-conjugated antibody specific for CRP was added to the wells after any unbound compounds were removed. Avidin-conjugated Horseradish Peroxidase (HRP) was added to the wells after washing. A substrate solution was then added to the wells after a wash to eliminate any unbound avidin-enzyme reagent, and color developed in proportion to the amount of CRP bound in the first stage. The color development was stopped and the intensity of the color was measured using the methods of Nnodi *et al*[14] and Abert *et al.*[15]

Histological processing and staining
On the 29th day, the animals were sacrificed while under halothane anesthesia, and the organs were removed, and rinsed in physiologic saline before being blotted with Whatman's number-1 filter paper. Organs were weighed, examined for obvious damage and necrotic changes, and then fixed in 10% buffered formal saline for 72 hours. The tissues were then processed using a Leica TP 1020 automatic tissue processor, dehydrated in four different alcohol concentrations (70, 80, 90, and absolute alcohol), cleared in three different xylene concentrations, and embedded using an embedding console (Leica EG 1160). Using a rotary microtome (Leica RM 2125), the blocks were sectioned at a thickness of 5 m after they had solidified. The sections were then floated in a water bath and heated to 60^OC for 30 minutes. The tissues were cut into sections, cleaned in xylene, and hydrated (90, 80, and 70%) in alcohol. Hematoxylin staining, 1% acid alcohol differentiation, scot tap water bluing, eosin counterstaining, and mounting in dibutylphthalene polystyrene xylene were all applied to the sectioned tissues. Photomicrographs were produced with a MoticTM 9.0 megapixel microscope camera at ×400 magnifications.[19]

Analysis of Data
Data were analyzed using one-way analysis of variance (ANOVA) in the statistical package for social science (SPSS) version 20 and provided as mean standard deviation (SD). $P \leq 0.05$ was adopted as the threshold for significance

Results and Discussion

A chronic condition known as diabetes mellitus can develop when the pancreas fails to make enough insulin or when the body struggles to transfer glucose. A total of 422 million individuals had diabetes in 2014, up from 108 million in 1980. Diabetes-related premature mortality increased by 5% globally between 2000 and 2016. With a projected 1.5 million fatalities directly related to diabetes in 2019, diabetes was the tenth largest cause of mortality.[20] This study looked at how serum inflammatory indicators, hyperglycemia, and the histology of the pancreas, liver, and kidney in Streptozotocin-induced diabetic rats responded to *Gongronema latifolium* leaf extract in ethanol. Streptozotocin concentration of 65 mg/kg body weight was used to

cause diabetes mellitus in the animals (STZ). This antibiotic is frequently used in experiments to generate insulin insufficiency and hyperglycemia in mouse and rat models, with reports that it also causes the death of pancreatic beta cells.[20] According to the findings, the experimentally-induced group's blood glucose level was greater than that of the diabetic induction group without therapy. This demonstrates the powerful role Streptozotocin played in the induction of diabetes, which was consistent with studies done by authors[21,22,23.]

Effect of Gongronema latifolium leaves extract on mean blood glucose levels

The result of mean blood glucose levels of both diabetic-induced and treated rats is presented in Figure 1 for days 1, 3, 14, 21 and 28. The diabetic-induced group (DM) had a significant increase (P = 0.018) in mean glucose values when compared with the control (t = 3.892) (P = 0.018). There was a statistically significant reduction (P = 0.052) in the mean glucose levels of the *Gongronema latifolium* (GL) treated group (t = 2.731) (P = 0.052) when compared with the diabetic non-treated group. Insulin treated group (DM+ Insulin) showed a statistically significant reduction (P= 0.052) in plasma glucose compared with the diabetic group. Insulin treated group (DM+ Insulin) showed a statistically significant reduction (P= 0.052) in plasma glucose compared with the diabetic group. More so, an important feature observed in this study was the observed ameliorating effect of *Gongronema latifolium* on the mean blood glucose level in the treatment group when compared with the induced group. There was a gross reduction in the fasting blood glucose levels of the treatment groups. This result is supportive of the previous study of, [23, 24] conducted on humans on the effects of *Gongronema latifolium* on blood lipids, lipoproteins, and glucose values in adult Nigerians. The study showed a significant reduction in the levels of plasma glucose of subjects fed with *Gongronema latifolium* for six weeks compared with baseline values. A previous study carried out on animals also showed reduced plasma glucose levels in aqueous *Gongronema latifolium* extract,[25] further suggesting that *Gongronema latifolium* has hypoglycemic properties. The mechanism of action by which *Gongronema latifolium* produces hypoglycemia has been suggested to be mediated through the activation of hexokinase, phosphofructokinase, glucose-6-phosphate dehydrogenase, and inhibition of glucokinase in the liver.[25] When these enzymes are activated, glycolysis and glycogenesis proceed faster resulting in lowering blood glucose. Furthermore, the constituents of *Gongronema latifolium* such as essential oils, saponins, alkaloids, flavonoids, phenols, and glycosides amongst others have been implicated in the prevention of degenerative diseases like diabetes mellitus and hypertension[26,,27,28]

Effect of Gongronema latifolium leaf extract on serum interleukin-6 (IL-6)

The mean serum level of interleukin-6 of both diabetic and non-diabetic treated rats is presented in Figure 3. The results showed that the serum interleukin-6 levels of the rats treated with *Gongronema latifolium*(GL) only (3.47±0.09) t = 4.779, P= 0.0170, and those induced and treated with extract and insulin were lower compared to the control (5.2±0.06), and statistically significant. The diabetic control (8.8±0.13) was significantly higher (P = 0.004) compared to the normal control and treated groups. The results of inflammatory biomarkers showed a significant reduction of C-reactive protein in the extract- treated group when compared to the induced group.The reduction in the mean level of C-reactive protein and interleukin-6 of the extract- treated group could therefore be attributed to the flavonoid and phenol found in *Gongronema latifolium* extract. The phenol substance has a chemo-protective role in the reduction of inflammation, according to a prior study by[27] on the chemo-preventive properties of anti-inflammatory phytochemicals.[28] Flavonoid, another phytochemical found in *Gongronema latifolium*, has also been demonstrated to have anti-inflammatory properties.[29]

Effect of Gongronema latifolium leaf extract on serum interleukin-6 (IL-6)

Figure 2 displays the interleukin-6 mean serum levels of treated diabetic and non-diabetic rats. The findings demonstrated that animals treated with simply *Gongronema latifolium* (3.47±0.09) t = 4.779, P= 0.0170, and those induced and treated with extract plus insulin (5.2±0.06) had significantly lower serum interleukin 6 levels than controls (5.2 ±0.06). In comparison to the normal control and treated groups, the diabetic control group's (8.8±0.13) value was considerably higher (P = 0.004). When compared to the provoked group, the extract-treated group's C-reactive protein level significantly decreased, according to the results of inflammatory biomarkers.± Therefore, the drop in the group that received extract treatment's mean levels of C-reactive protein and interleukin-6 might be attributable to the extract's *Gongronema latifolium* extract contains phenol and flavonoid. The phenol substance has a chemo-protective role in the reduction of inflammation, according to a prior study by Edet *et al*[29] on the chemo-preventive properties of anti-inflammatory phytochemicals.[30]

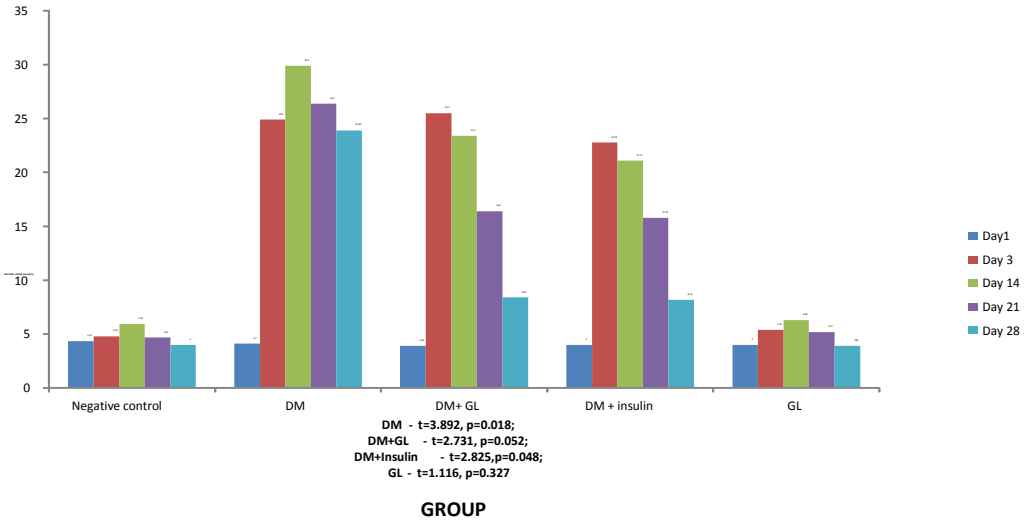

Fig. 1: Mean blood glucose levels of diabetic and non -diabetic treated rats

Flavonoid, another phytochemical found in *Gongronema latifolium*, has also been demonstrated to have anti-inflammatory properties.[31]

Effect of Gongronema latifolium leaf extract on C–reactive protein

Figure 4 displays the results of the serum C-reactive protein levels in diabetic and non-diabetic treated rats. When compared to the normal control group's (2.3±0.15) serum C-reactive protein levels, the *Gongronema latifolium*(GL) only group's (2.4±0.18) levels were higher, although this difference was not statistically significant (t=0.367, df(3), P = 0.738). The outcomes demonstrated that, in comparison to the levels in the diabetic control group (DM, 5.400.22), the serum C-reactive protein levels of the GL- treated diabetic rats (2.63±0.12, t=4.270), (P =0.024), and insulin-treated diabetic rats (2.50±0.10, t = 47.217, P = 0.001) groups were lower and statistically significant. According to the findings, *Gongronema latifolium* leaf extract has anti-inflammatory activities.[28] C-reactive protein and interleukin-6 levels were significantly lower according to the results of inflammatory biomarkers when compared to the induced group, the extract-treated group. Furthermore, it has been demonstrated that the phytochemical flavonoid, another component of G*ongronemalatifolium*, has anti-inflammatory properties.[29, 34]

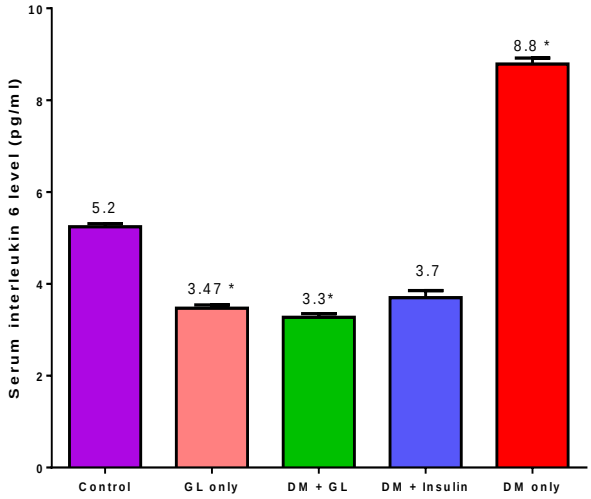

Figure 2: Mean serum levels of interleukin-6 in diabetic and non-diabetic treated Rat.

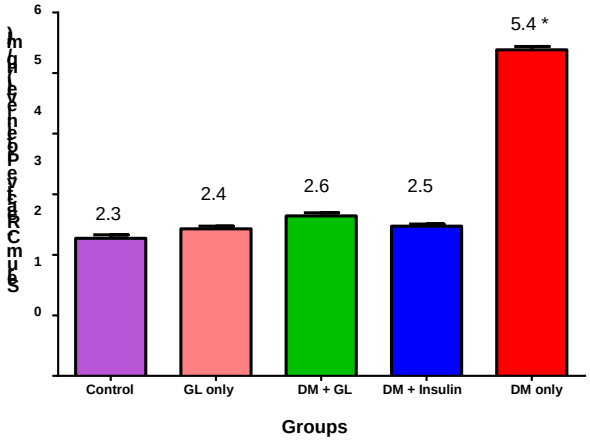

Figure3: Serum C-reactive protein levels in diabetic and non-diabetic treated rats.

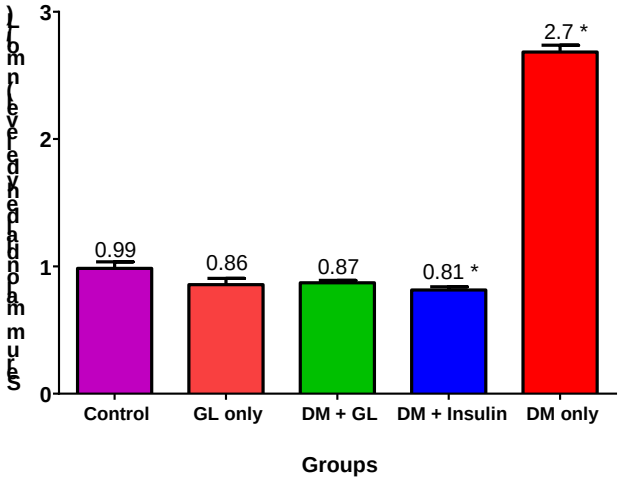

Figure 4: Serum malondialdehyde level in diabetes and non-diabetes-treated rats
F =0.347, P=0.108, NS

Effect of Gongronemalatifolium leaf extract on serum malondialdehyde level

Figure 4 displays the serum malondialdehyde level (MDA) results for rats treated for diabetes and rats not treated for diabetes. In comparison to the normal control (0.98±0.13), the serum malondialdehyde levels of GL alone (0.860±12, t= 0.414, P = 0.700), DM+GL (0.87±0.04, t = 1.441, P = 0.223), and DM+Insulin (0.81±0.06, t = 2.101, P = 0.126) did not differ statistically. In comparison to the normal control (0.98±0.13), the serum MDA level of the DM alone group (2.7±0.11) was statistically substantially (t = 17.771, P = 0.001) higher. A marker called malondialdehyde is utilized to look into the occurrence of oxidative stress and tissue damage. When compared to all other treatment groups (extract and insulin), the mean level was normal in the control group and significantly lower when compared to the group that had diabetes induced. Compared to the insulin-treated group receiving the usual medication, this impact was more significant in the extract-treated group. The new study supports the previous study's finding that *Gongronema latifolium* extract possesses antioxidant properties.[30] Additionally, a study from [29] shows how the antioxidant activity of flavonoids, one of *Gongronema . latifolium*'s ingredients, might reduce the oxidative damage produced by reactive oxygen species (ROS). All of these pieces of evidence support the study's conclusions and support the extract's beneficial effects on oxidative stress inhibition.

Effects of Gongronema latifolium on the histomorphology of the pancreas

For histomorphological changes, the pancreas photomicrographs from the normal, diabetic, and *Gongronema latifolium* treatment groups were analyzed. Endocrine and exocrine components were normal according to the control group's findings. The exocrine portion had a lumen filled with mucinous secretion and conspicuous acini lined by cuboidal epithelium. The islet of Langerhans had intact capsule and cords of an oval-to-round cells spaced apart by blood capillaries, made up the endocrine portion. In the healthy control rats, the cells were centralized and heavily populated (Plate 1. CON). The pancreas in the diabetes mellitus group has thin, undamaged capsules surrounding its sparsely populated Langerhans islet cells. The sparse cells were organized in blood capillaries that divide into cords and nests. The cells were gathered near the periphery and the nuclei were very basophilic with a thin border of cytoplasm. Additionally, it revealed deteriorated pancreatic islet cells with enlarged and clogged blood channels, as seen in (Plate 1. DM). In the insulin-treated group, the islet and dilated cells, as well as the acini cells had fewer cells (Plate 1.DM+ IN). The photomicrograph of the pancreas from diabetic mice receiving *Gongronemalatifolium* treatment revealed prominent Langerhans islet

cells encased in an intact capsule. The *Gongronemalatifolium* alone treated group had uniformly dispersed cells with normal shape seen in Plate 1. GL., while the densely populated oval-to-round cells were divided by congested capillaries (DM+ GL). This finding is consistent with reports of WHO[13]. This also suggests, as supported by studies of[16,23] that G*ongronemalatifolium* induces regeneration of the pancreatic beta cells that were destroyed by oxidative stress.

Histological effects of Gongronema latifolium on the liver

Hepatocyte plates extending outward from a central vein were visible in the control group. According to Plate 2.CON, the hepatocytes were divided into sinusoidal gaps, and the cells contain conspicuous nuclei with a regular form and prominent nucleoli. The histology of the liver in the diabetes mellitus group revealed clogged portal veins and mild portal mononuclear cellular infiltrates with intact limiting plates. The hepatocytes had large cytoplasm and nuclei. There was a slight dilation of the intervening sinusoidal spaces. Hepatocyte plates radiated outward from the central veins, which were visible in Plate 2.DM. A photomicrograph of the liver after treatment with insulin and G*ongronema latifolium* revealed a prominent central vein and hepatocyte plates that were radiating from it. Hepatocytes in the centro-lobular sections of the liver were enlarged with strong hepatic outlines toward the portal area. The diabetic group treated with G*ongronema latifolium* (DM+ GL) and diabetic group treated with insulin (DM + IN) had conspicuous enlarged nuclei. It is well established that oxidative stress damages tissue in both animal and human models. This study found that *Gongronema latifolium* may repair liver damage caused by the development of diabetes. This conclusion is consistent with other research that found the extract to have hepato-protective qualities.[4] Previous investigations claimed that *Gongronema latifolium* leaf extracts in methanol have hepatotoxic effects, hence extreme caution must be used while taking the plant's extract as a natural treatment for any condition.[32]

Histological effects of *Gongronema latifolium* on the kidney

The kidney of the control group displayed normal renal tubules and glomeruli. Normal cellular mesangial and Bowman space were features of the glomeruli. The interstitia between the renal tubules were sparse, while the lining epithelium, which is made up of cuboidal to columnar cells, was intact (Plate 3.CON). In the diabetes mellitus group, the kidney's histology revealed atrophic glomeruli along with bleeding into the mesangial matrix and the death of the mesangial cells. Focal segmental sclerosis was found. As seen in Plate 3. DM, the renal tubules were prominent and bordered by cuboidal to columnar epithelial cells. The kidneys of the diabetic rats treated with *Gongronema latifolium* and regular insulin were photographed, and the photomicrograph revealed large glomeruli with a cellular mesangial matrix made up of proliferating cells unique to the Bowman space and there were numerous tubules. There was no difference between the renal vasculature and control. The kidneys of diabetic rats treated with *Gongronema latifolium* and regular insulin were photographed, and the photomicrograph revealed large glomeruli with a cellular mesangial matrix made up of proliferating cells. The renal tubules were visible, and the Bowman space was defined. When compared to the control, there was no difference in the renal vasculature. The findings of this study clearly demonstrate that *Gongronema latifolium* has more nephron-protective properties than insulin.[33]

Conclusion

In addition to reversing the organ damage resulting from the induced diabetes, the use of *Gongronema latifolium* resulted in a decrease in mean blood glucose, interleukin-6, C-reactive protein, and malondialdehyde levels. *Gongronema latifolium* reduced oxidative stress-related necrosis and cellular damage to the pancreas, kidney, and liver.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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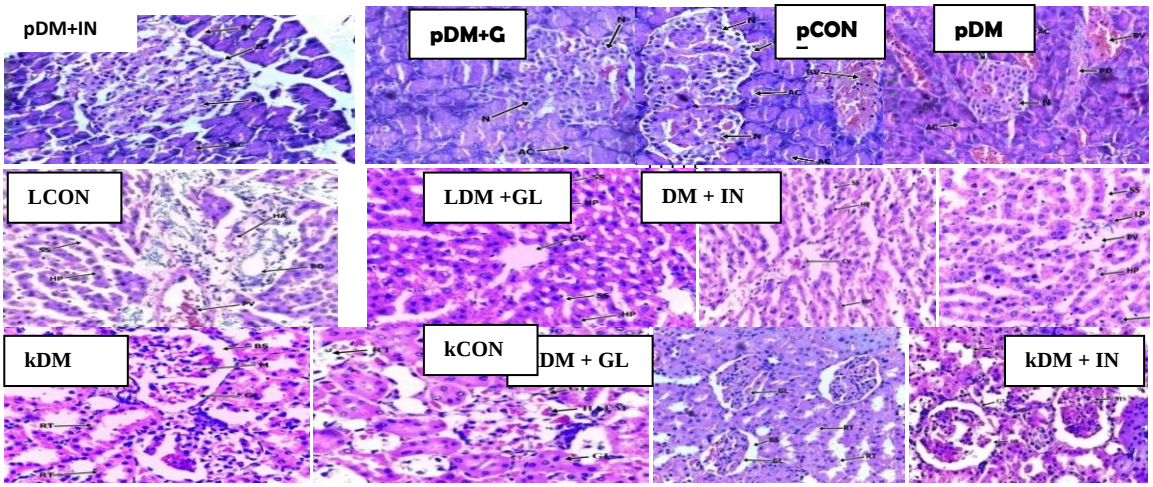

Plate 1: *Photomicrograph of pancreas(p) , liver(L) and kidney(k) tissue respectively stained with haematoxylin and eosin stain x400 magnification. CON = Normal rat, DM: Diabetic rat,* DM+IN *= diabetic rats treated with Insulin,* DM+GL *= diabetic rats treated with Gongronema latifolium leaves extract. N= nucleus; AC = Acini cells; IL = Islet cells of Langerhans; BV=blood vessel; PD=Pancreatic duct.* HP=Hepatocytes; SS=sinusoidal space; CV=Central Vein; HA=Hepatic artery; BD=Bile duct .. GL=Glomerulus; BS=BowmanCapsule; MS=Mesangium; RT=Renal Tubules; BV= Blood Vessels.

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