JIPBS

Research article

# Nephroprotection against streptozotocin diabetes is more effective in combined than single leaves extracts of *Gongronema Latifolium* and *Ocimum Gratissimum L.*

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**Abstract**

This study investigated the nephroprotective potency of combined leaves extracts of *Gongronema latifolium* (GL) and *Ocimum gratissimum L.* (OG) against streptozotocin-induced diabetic rats. Thirty-six male albino rats were divided into 6 groups of 6 rats each. Groups A and F received placebo treatment and each served as diabetic and normal controls, B, C and D respectively received 200mg/kg b.w GL and OG and 100mg/kg b.w. each of combined GLOG and E received 5 IU/kg b.w insulin (subcutaneously). After 28 days of treatment, animals were sacrificed and serum collected for analysis of glucose and kidney function indices. From the results, serum concentrations of sodium, chloride, bicarbonate and phosphorus were significantly (p<0.05) reduced while potassium level increased significantly in DC compared to NC and treatment groups. The altered concentrations of these electrolytes observed in single extract treatments were effectively modulated in combined. Also, the extracts significantly (p<0.05) reduced STZ –induced elevation in serum concentrations of glucose, creatinine, urea BUN and uric acid in DC compared to NC. Conclusively, the kidney lesions salvaging effect and the amelioration of diabetes associated perturbations of kidney function indices were more effective in combined than single extracts, hence revealing a positive synergistic interactions of the two extracts.

**Keywords:** Diabetes, rats, *Gongronema latifolium*, *Ocimum gratissimum L.,* kidney

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## 1. Introduction

Diabetes mellitus is a metabolic disease characterized by persistent hyperglycaemia coupled with increased thirst, polyuria, polyphagia, and weight loss due to absolute or relative lack of insulin [1]. It is a serious metabolic disorder with micro and macro vascular complications that results in significant morbidity and mortality [2]. Studies have shown that individuals with diabetes have a higher incidence of liver and kidney function abnormalities, as well as formation of free radicals due to glucose oxidation, non-enzymatic glycosylation of proteins and subsequent oxidative

degradation of glycated proteins, leading to a decline in antioxidant defense mechanisms and damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance [3,4]. Virtually every organ system participates in the maintenance of fluid balance and /or is adversely affected by imbalances. In many disease states, impaired fluid intake, excessive fluid losses, or organ damage and dysfunction results in a state of altered fluid and electrolyte balance [5]. Electrolytes are crucial to the function of every cell in the body. Under conditions where a disease such as diabetes upsets metabolic function, the body's electrolyte control system breaks down. It is a known fact that kidney function is compromised in uncontrolled diabetes mellitus. The kidney functions may be assessed from the level of some electrolytes (such as $K^+$ $Na^+$, $Cl^-$) and metabolites (such as creatinine, urea and blood urea nitrogen) in the plasma [6,7]. Renal dysfunction may be caused by several diseased conditions and exposure to certain reactive or toxic metabolites [6,8,9]. Renal fibrosis are the principal process involved in the progression of chronic kidney disease [10], urethral obstruction, malignant hypertension, severe diabetic condition, or chronic exposure to heavy metals [11]. Once the renal tissues are damaged, the overall functionality of the kidneys may be compromised. Kidney function is assessed in clinical practice to screen for kidney disease, to adapt dosage of medications for renal clearance, and to follow the evolution of known kidney dysfunction. Glomerular filtration rate (GFR) is a useful index to assess the kidney function [12]. Medicinal plants contain several different pharmacologically active compounds that may act individually, additively or synergistically to improve health and they

are widely preferred owing to their availability, affordability (cost effectiveness) and increased safety [13]. A combination of herbs or phytochemicals from more than one source has proved more useful and beneficial in the management of various ailments because the combined secondary metabolites synergistically potentiate biological effects with minimum side effects [14]. *Gongronema latifolium* (*G. Latifolium*) of the family Asclepiadaceae, locally known as 'utazi' in the Igbo dialect of the eastern Nigeria is a tropical rainforest plant primarily used as a spice and vegetable in the traditional folk practice [15]. Phytochemical evaluation of the plant has shown that it is rich in essential oils, alkaloids, tannins, phytates, flavonoids saponins, pregnanes and oligosaccharides amongst others [16,17]. In the southern part of Nigeria it is used traditionally in the treatment of malaria, diabetes and hypertension as well as a laxative. *Ocimum gratissimum* L. (Labiatae) is a native of Africa and Asia, but is now distributed to other parts of the World including the United States of America [18]. In Nigeria, *O. gratissimum L.* is described by different local names: Daidoya (Housa), Nchunwu (Igbo), Efnrin (Yoruba), Nton (Ibibio) [19] but it is popularly known as "scent leaf" in most parts of the country. Studies have shown that the leaf extract of O. *gratissimum L.* contains potent bioactive components (essential oils) made up of eugenol, citral, linalool, charvicol, thymol, gerianol, triterpenoids, saponins, alkaloids, etc. [18,20,21]. These phytochemicals possess antibacterial [22], antifungal [23], antinociceptive [24], antihypertensive [25-26], antidiabetic [27], antidiarrhoeal [28, 29], antioxidant [30], insecticidal [31] and antihelmintic properties which justify its high medicinal use in folk medicine [32]. Comparatively, the combined extracts of

*Gongronema latifolium* and *Ocimum gratissimum L.* were reported to significantly possess antihyperglycemic, antihyperlipidemic and antioxidative properties [33, 34] against STZ diabetic rats and potentiate *in vitro* free radical scavenging [37] than single extracts. Also, antinephrotoxic and hepatoprotective potentials of these leaves extract against carbon tetrachloride induced toxicity in rats were found to be more effective in combined treatments than single [36].

In this study, nephroprotective capacity of combined compared to single leaves extracts of *Gongronema latifolium* and *Ocimum gratissimum L.* against STZ-induced diabetic rats were investigated.

## 2. Materials and Methods
### Collection and Preparation of Plant Materials

Fresh but matured leaves of *Gongronema latifolium* and *Ocimum gratissimum* were collected from Atimbo, Akpabuyo Local Government Area of Cross River State. They were both identified and authenticated in the Department of Botany, University of Calabar, Calabar Nigeria. 1kg each of *Gongronema latifolium* and *Ocimum gratissimum* were thoroughly washed with clean tap water to remove dust particles and debris and shade dried. The dried plant materials were grounded into powder with a KENWOOD electric blender (KENWOOD LTD. ENGLAND). The powdered samples were each kept in a glass container with a plastic screwed cap and kept for 12 hrs, the extract was thereafter concentrated *invacuo* at 37-56°C using a rotator evaporator. The concentrates were allowed open in a water bath (40°C) for complete ethanol removal. The dried extracts were refrigerated at 2-8°C until required for use. The concentration of the extract was determined by drying a known volume and measuring the dry weight.

### Animals

Thirty-six (36) male albino rats of Wistar strain weighing between 164-258g were obtained from the animal house of the College of Health Sciences University of Uyo. Upon arrival, the animals were allowed to acclimatize for two weeks in the Biochemistry departmental animal house facility, University of Calabar where the experiment was later carried out. The animals were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions.

### Induction of Experimental Diabetes

Prior to diabetes induction, the rats were subjected to 12hr fast and then diabetes was induced by intraperitoneal injection of 65mg/kg b.w (Ugochukwu and Babady, 2003). Streptozotocin (STZ) (Sigma St. Louis, MO, USA) reconstituted in 0.1M Na citrate buffer (pH 4.5). Seven days after, diabetes was confirmed in STZ treated rats with a fasting blood sugar concentration ≥ 200mg/dl. The blood glucose was determined using One Touch Glucometer with blood obtained from the tail vein of the rats. The diabetic rats were then divided randomly into the different groups.

### Experimental Design and Treatment of Animals

36 male albino Wistar rats were divided into six groups of six rats per group and treated (Table 1). The plant extracts reconstituted in distilled water (vehicle) were administered via oral gastric intubation twice per day (7.00 am and 7.00 pm). The dosage of the extract was determined from preliminary studies in

boratory. Insulin was administered subcutaneously (S.C) once daily

**Table 1. Experimental design**

| Group | No. of animals | Treatment |
|-------|----------------|-----------|
| A | 6 | Placebo (Diabetic Control) |
| B | 6 | GL extract (200mg/kg bw) |
| C | 6 | OG) extract (200mg/kg bw) |
| D | 6 | GL (100mg/kg) + OG (100mg/kg) |
| E | 6 | Insulin (5 IU/kg bw) |
| F | 6 | Placebo (normal control) |

**Collection of kidney and blood for serum preparation**

After 28 days of treatment, the animals were sacrificed by cervical dislocation. The kidney was excised immediately and thoroughly washed in ice – cold saline and was preserved in 20% formalin for histopathological study. The blood was collected through cardiac puncture using a sterile needle to pierce through the heart and emptied into a sterile test tube containing no anticoagulant and was allowed to clot and centrifuged at 3000rpm at 4°C for 10 mins. Serum was separated with sterile syringes and needles and stored frozen until used for the analysis of glucose, urea, BUN, uric acid, creatinine, $K^+$, $Cl^-$, $Na^+$, P and $HCO_3^-$.

**Serum glucose determination**

Serum glucose was estimated using Dalab reagent kits, by the principle of the glucose oxidase method. In this principle, glucose oxidase oxidizes glucose to gluconic acid, and hydrogen peroxide formed as a byproduct. The peroxide whose concentration is in proportion to glucose in the sample develops a quantifiable colour via 4-aminophenazone in the presence of a peroxidase.

**Determination of serum urea and blood urea nitrogen**

Urea in serum was estimated based on the endpoint colorimetric method using automatic analyzer (Olympus AU2700, Olympus Co., Tokyo, Japan). In this method urease enzyme hydrolyzes urea to ammonia and carbon dioxide. The ammonia so formed reacts with alkaline hydrochloride and sodium salicylate in the presence of sodium nitropruside to form a coloured chromophore, which was spectrophotometrically measured.

**Serum creatinine assay**

The concentration of serum creatinine was measured based on the reaction of creatine with alkaline solution of sodium pirate to form a red complex. [38]. The red coloured complex which was proportional to the concentration of creatinine in the sample was measured spectrophotometrically.

**Determination of sodium, potassium, chloride and bicarbonate ions and phosphorus inorganic in serum**

The serum concentrations of sodium, potassium and chloride ions were determined by colorimetric methods using kit obtained from TECO diagnostics, Anaheim, USA based on [39]. Sodium is precipitated as the salt, sodium, magnesium

Urany1 acetate, with the excess uranium then being reacted with ferrocyanide to produce a chromophore whose absorbance (colour intensity) at 550nm varies inversely as the concentration of sodium in the test specimen.

Serum potassium was determined by direct measurement of absorbance at 500nm of a colloidal suspension formed when potassium in the serum sample mixed with sodium tetraphenylboron. The turbidity of the solution is directly proportional to the concentration of potassium in the sample in the range of 2-7mEq /L.

Chloride ions in the sample react with mercuric thiocyanate to release free thiocyanate ions, which forms a coloured complex with ferric ions with the colour intensity proportional to the chloride ion concentration in the sample:

$$6\,Cl^- + 3\,Hg\,(SCN)_2 \longrightarrow 3HgCl_2 + 6(SCN)^-$$

$$6(SCN) + 2Fe^{3+} \longleftrightarrow 2Fe\,(SCN)_3$$
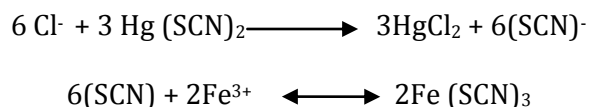
Serum bicarbonate ion concentration was determined by titrimetric method [40]. 1ml of 0.01N hydrochloric acid was added to a fresh dilute serum sample.shaking the sample thoroughly expelled the carbondioxide formed. The hydrogen ions that remained were titrated against 0,01N sodium hydroxide using two drops of phenol red until a pink end point was obtained. The concentration of bicarbonate ion in serum was calculated,

Phosphorus inorganic in serum was determined using DIALAB assay kits. In acid medium phosphate ions react with ammonium molybdate to form a yellow phosphorus molybdate complex with absorption maximum at 340nm. Absorption is in proportion to the concentration of in organic phosphorus in the sample.

**Histopathological studies**
After 28 days of treatment, the kidney from each group was removed. It was immediately blotted using filter paper to remove traces of blood and then weighed with analytical balance. Thereafter, the tissue was suspended in 10% formalin after washing with normal saline for fixation preparatory to histological processing. Kidney tissue was fixed in neutral formalin solution for 48 hrs dehydrated by passing through a graded series of alcohol embedded in paraffin blocks. 4 μm thick sections were prepared using a semi-automated rotator microtome

**Statistical analysis**
The results were analyzed for statistical significance by one-way ANOVA using the SPSS statistical program and least square test (LSD) between groups using MS excel programme. All data were expressed as mean + SEM. P value <0.05 was considered significant.

**3. Results and Discussion**
Presented in the table 2 is the result of changes in selected serum electrolyte concentrations of untreated normal and diabetic control rats and diabetic rats treated for 28 days with extracts of GL, OG singly and combined and insulin. Serum concentrations (mEq/L) of sodium (Na:102. 92±0.08), chloride (Cl$^-$:70.93±0.03), bicarbonate (HCO$_3^-$:9.78±0.01) and phosphorus (P:3.10±0.17) were significantly (p<0.05) reduced while potassium (K$^+$) level increased (K:10.30 ±0.01) significantly in untreated STZ-induced diabetic (DC) rats compared to normal control. Treatment with extracts and standard drugs significantly (p<0.05) increased the concentrations of these electrolytes (except K$^+$ which decreased significantly) when compared to DC groups.

**Table 2. Serum electrolytes of treated and untreated diabetic rats.**

| Group/ treatment | $K^+$ (mEq/L) | $Na^+$ (mEq/L) | $Cl^-$ (mEq/L) | $HCO_3^-$ (mEq/L) | P (mEq/L) |
|---|---|---|---|---|---|
| DC | 10.30 ±0.01* | 102.92 ±0.08* | 70.93 ±0.03* | 9.78 ±0.01* | 3.10 ±0.17* |
| $D_{GL}$ | 8.77 ±0.01*,a,b,c | 116.34 ±0.01*,a,c | 83.72 ±0.01*,a,b,c | 13.07 ±0.18*,a | 5.97 ±0.02*,a,b,c |
| $D_{OG}$ | 7.80 ±0.01*,a,b,c | 119.80 ±0.07*,a,b,c | 96.32 ±2.19*,a,b,c | 13.55 ±0.03*,a | 5.65 ±0.04*,a,b,c |
| $D_{GLOG}$ | 6.07 ±0.15*,a | 129.37 ±0.14*,a | 100.63 ±0.21*,a | 14.62 ±0.03*,a | 7.45 ±0.10*,a |
| $D_I$ | 7.01 ±0.13*,a,b | 115.01 ±0.25*,a,b | 102.34 ±0.35*,a | 14.55 ±0.03*,a | 5.03 ±0.46*,b |
| NC | 5.56 ±0.01 | 122.88 ±0.01 | 104.16 ±0.06 | 14.98 ±0.05 | 8.93 ±0.01 |

*p<0.05 vs NC; a = p<0.05 vs DC; b = p<0.05 vs $D_{GLOG}$;  c = p<0.05 vs $D_I$ Values are expressed as mean ± SEM, n = 6.

DC= Diabetic control;

$D_{GL}$= Diabetic treated with the *Gongronema latifolium extract*

$D_{OG}$ = Diabetic treated with the *Ocimum gratissimum* extract;

$D_{GLOG}$ = Diabetic treated with combined extracts of *Gongronema latifolium L.* and *Ocimum gratissimum L.*

$D_I$= Diabetic treated with insulin;

NC= Normal control

The altered concentrations of these electrolytes (hyperkalemia, hyponatremia, hypochloremia, hypophosphatemia and decreased bicarbonate level) observed in single extract treated groups were effectively modulated in combined extract treatment and their concentrations compared well with normal control values. Diabetes is characterized by increased volume and metabolites excretions via the kidney, usually in excess of normal thresholds. This usually gives rise to derangement in homeostatic balance with respect to electrolytes. Potassium is largely an intracellular ion and its distribution across the cell membrane plays a critical role in the maintenance of cardiac and neuromuscular excitability. Changes in $K^+$ concentrations which alter the ratio of intracellular to extracellular potassium alter membrane potential [5]. In general, hypokalemia increases membrane potential, causing hyperexcitability [5]. The significant (p<0.05) increase in the concentrations of potassium (hyperkalemia) in GL and OG extract treated rats was reversed in combined extract treatment, hence revealing a striking complementary action. Metabolic acidosis is associated with hyperkalemia because increased hydrogen ions are available for sodium exchange in the renal tubules at the expense of potassium ions and

hence result in increase retention of potassium in serum. Impaired glomerular filtration amidst hyponatremia reduces the amount of sodium ions available in renal tubule for potassium exchange and therefore contributes to the elevation of serum $K^+$ concentration. The decreased serum concentration of $K^+$ in insulin treated rats might not be unrelated to the enhancement by insulin of cellular uptake of $K^+$ along glucose from the extracellular fluid compartment. The significant ($p<0.05$) decrease in the concentration of $Na^+$ (hyponatremia) and $Cl^-$ (hypochloremia) in diabetic control rats relative to normal control could be attributed to osmotic diuresis with subsequent loss of water (dehydration) and electrolytes induced by glycosuria [40]. This ketoacidosis is a prevalent feature which leads to ketonuria. An attempt by the kidney to buffer the urine decreases the alkaline metals including $K^+$ and $Na^+$ in serum [41].

Marked hyperglycemia associated with diabetes mellitus or the administration of glucose at an excessive rate generally produces a hyponatremia. As glucose concentration increases in the extracellular fluid (ECF), osmotic forces are generated that result in movement of cellular water into the ECF diluting serum Na concentration. The hyponatremia and hypochloremia became revered with extracts treatments. This presents a good potential in diabetic management because derangement in electrolytes is what usually leads to hypovolemic shock via depressed CNS leading to death in uncontrolled diabetes [42]. Acidosis as evidently shown in the decreased concentration of bicarbonate in the untreated diabetic rats may be as a result of increased anaerobic catabolism of glucose with subsequent generation of large amounts of lactic acid [43]. The decreased serum concentration of phosphorus (hypophosphatemia) in untreated diabetic rats is again not unrelated to concomitant loss together with other electrolytes incidental to dehydration [41,44]. Phosphorus is predominantly an intracellular electrolyte, pivotal in tissue metabolism of glucose and carbohydrate in general. Its concentration increased significantly following extract treatment. From the results, the effect of administration of GL and OG on serum electrolytes of STZ diabetic rat model suggests that increased serum levels of K (hyperkalemia) and bicarbonate, and decreased concentrations of $Na^+$ (hyponatremia), Cl (hypochloremia) and P (hypophosphatemia) in diabetes could be significantly reversed following single GL and OG extracts treatment with the combined extract displaying a positive synergistic effect.

Table 3 presents the effect of treatment on the levels of glucose, creatinine, urea, blood urea nitrogen (BUN) and uric acid in serum of untreated and treated diabetic rats. There were significant ($p<0.05$) increases in serum glucose, creatinine, urea BUN and uric acid in diabetic control rats compared to normal control rats. These serum parameters decreased significantly ($p<0.05$) in diabetic treated groups when compared to diabetic control (DC). The combined extracts treated group exhibited significant ($p<0.05$) reductions in these parameters as compared to single extracts treated groups. Streptozotocin induction was predisposing factor to impairment of renal functions as judged by significant increases in these serum parameters. The significant increases in serum creatinine, urea, and BUN and uric acid concentrations in DC may be linked to the interactions of STZ with renal tissues to impair the glomerular functions. However, treatments with the

extracts singly and in combination effected significant reductions. These decreases imply that renal function impairment associated with a diabetogenic agent might be averted or significantly reversed by a 28-day oral administration of the crude leaves extracts of GL and OG singly and in combination.

**Table 3. Serum concentrations of glucose, creatinine, urea, BUN, and  uric acid of treated and untreated diabetic rats.**

| Group/ treatment | Glucose (mEq/L) | Creatinine (mg/dl) | Urea (mg/dl) | BUN (mg/dl) | Uric acid (mg/dl) |
|---|---|---|---|---|---|
| DC | 351.52 | 1.09 | 30.22 | 45.83 | 4.88 |
|  | ±9.89* | ±0.03* | ±0.08* | ±0.51* | ±0.17* |
| $D_{GL}$ | 86.86 | 0.82 | 27.73 | 33.95 | 2.97 |
|  | ±1.53*,a,b | ±0.00*,a,b,c | ±0.06*,a,b,c | ±0.28*,a | ±0.02*,a,b,c |
| $D_{OG}$ | 84.58 | 0.83 | 27.09 | 33.60 | 2.88 |
|  | ±0.33*,a,b | ±0.01*,a,b | ±0.10*,a,b | ±0.23*,a | ±0.02*,a,b,c |
| $D_{GLOG}$ | 42.48 | 0.72 | 25.41 | 28.61 | 1.56 |
|  | ±0.21a | ±0.00*,a | ±0.11*,a | ±0.35*,a | ±0.01*,a |
| $D_I$ | 83.14 | 0.86 | 27.10 | 32.57 | 5.03 |
|  | ±0.33*,a,b | ±0.01*,a,b | ±0.01*,a,b | ±0.23*,a | ±0.46*,b |
| NC | 40.12 | 0.29 | 23.75 | 27.82 | 2.08 |
|  | ±1.30 | ±0.00 | ±0.09 | ±0.52 | ±0.01 |

*p<0.05 vs NC; a = p<0.05 vs DC; b = p<0.05 vs $D_{GLOG}$;  c = p<0.05 vs $D_I$ Values are expressed as mean $\pm$ SEM, n = 6.

DC=     Diabetic control;
$D_{GL}$=     Diabetic treated with the *Gongronema latifolium extract*
$D_{OG}$ =   Diabetic treated with the *Ocimum gratissimum* extract;
$D_{GLOG}$ = Diabetic treated with combined extracts of *Gongronema latifolium* and *Ocimum gratissimum L.*
$D_I$=     Diabetic treated with insulin;
NC=     Normal control

In this study, the significant (p<0.05) increase in serum creatinine, urea, BUN and uric acid concentrations compared to normal control suggest impairment in the diabetic kidney. Urea and creatinine are considered as suitable prognostic indicators of renal dysfunction and kidney failure for any toxic compounds [45]. Urea is the end-product of the protein metabolism, and it is synthesized in the liver from the ammonia produced in the catabolism of amino acids. It is transported by the blood to the kidney from where it is excreted [46]. Increased blood urea production in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins [47]. Diabetic hyperglycaemia induces elevation of the plasma levels of urea and creatinine, which are considered significant markers of renal dysfunction.

Although the specific mechanism for the renal dysfunction inferred from this study is yet to be understood, it might be suggested that STZ coupled with their metabolites on interaction with the renal tissue, possibly resulted in conditions such as glomerulonephritis, nephritis, nephrosclerosis polycystic kidneys and tubular necrosis [48]. Deficiency of insulin, an anabolic hormone and inability of glucose to reach the extra-hepatic tissues, stimulates gluconeogenesis as an alternative route of glucose supply [49]. Gluconeogenesis is sustained by increase proteolysis which releases free glucogenic amino acids circulated in plasma. These are deaminated in the liver with the consequence of increased urea in blood. As glucose is cleared from serum and insulin effect probably reintroduced proteolysis reclines through hormonal stimulation [49], hence decrease urea concentration in blood.

Creatinine is the catabolic products of creatinine phosphate which is used by the skeletal muscle. It is a metabolite of muscle – creatine, whose amount in serum is proportional to the body's muscle mass. The amount of creatinine is usually constant, so that elevated levels indicate diminished renal function only, since it is easily excreted by the kidneys [50].

Uric acid is formed by the breakdown of purine and by direct synthesis from 5-phosphoribosyl pyrophosphate and glutamine. Population- based studies have shown that hyperuricemia is an independent risk factor for cardiovascular disease (CVD). This association has been found to be particularly robust among individuals at high risk for CVD including those with obesity, hypertension, diabetes and renal disease [51]. The actual mechanism of hyperuricemia is unknown, but it has been observed that compensatory hyperinsulinemia in insulin resistant individuals impose an antiuricosuria effect on the kidney [52,53]. The association of uric acid with diabetes mellitus has also been explained on the basis of genetic predisposition [54,55]. Hyperuricemia may result in acute gouty arthritis, nephrolithiasis and nephropathy [56, 57]. Hyperinsulinemia reduces the renal excretion of uric acid and sodium. Hyperuricemia resulting from euglycemic hyperinsulinemia may precede the onset of type 2 diabetes, hypertension, coronary artery disease, and gout in individuals with metabolic syndrome [57].

Diabetic kidneys are prone to impairment, hence contributing to electrolytes perturbation and the increased serum urea, creatinine, BUN and uric acid. Treatments with extracts and insulin caused significant decreases (p<0.05) in these indices compared to DC, hence suggesting the protective effects of these extracts and standard drug against STZ induced renal dysfunction and kidney failure.

Figures 1-6 present the results of histology of kidney tissue of both treated and untreated diabetic rats. In the untreated normal control rats (figure 1), histological examination revealed a morphologically normal kidney. The renal tissue showed glomeruli having normal mesangial cellularity, and tubules having epithelial tufts. The interstitium was normal. The photomicrograph of a typical kidney section of diabetic untreated rats (figure 2) revealed abnormal condition. Untreated diabetic rats showed renal tissue with marked microscopic changes like multifocal clarifications and vacuolations compared to normal control kidney. In morphometric studies, we observed significant atrophy of glomerular capillaries with Bowman's space dilated in untreated diabetic rats. In diabetic rats treated with GL (figure 3), the renal tissue showed glomeruli with moderate increase in mesangial

cellularity and normal tubules with focal areas of haemorrhage in the interstitium. Here the incidence and intensity of tubular vacuolations as well as other degenerative structures were much lower compared to DC. The diabetic OG treated rats (figure 4) revealed renal tissue showing normal glomeruli and tubules as well as interstitium with focal areas of recent haemorrhages. Also, diabetic rats treated with GLOG (figure 5) showed in their renal tissue glomeruli with moderate increase in mesangial cellularity, normal tubules and interstitium. Their renal tissues showed normal glomeruli, tubules and interstitium. The diabetic rats treated with insulin had renal tubule showing normal glomeruli, tubules and interstitium (figure 6).



**Figure 1. Photomicrograph of kidney of normal control rats given placebo treatment (x 400). T= tubules, G=glomerulus, I= interstitium**
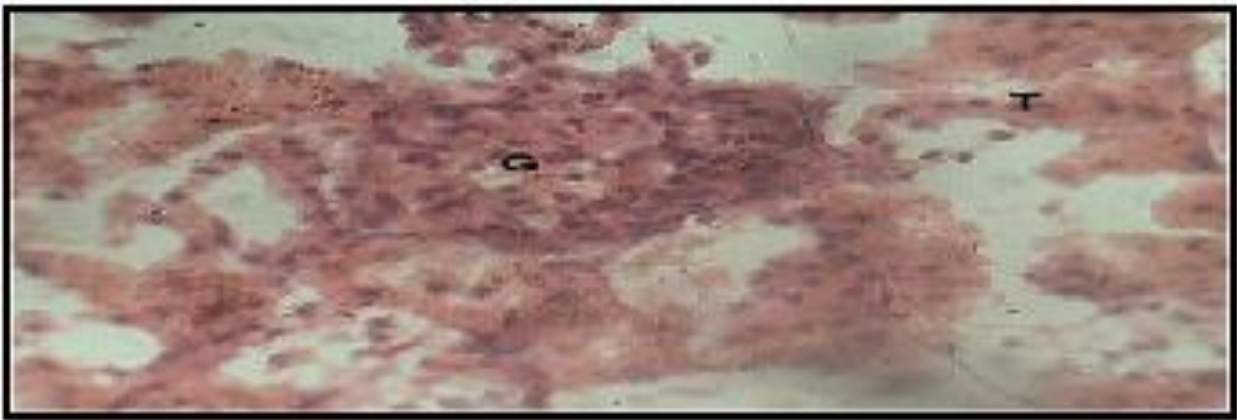


**Figure 2. Photomicrograph of kidney of diabetic control rats given placebo treatment (x 400). T= tubules, G=glomerulus, I= interstitium.**

**Figure 3. Photomicrograph of kidney of diabetic hypertensive control rats treated with 200mg/kg b.w. GL (x 400). T= tubules, G=glomerulus, I= interstitium**.



**Figure 4. Photomicrograph of kidney of diabetic rats treated with 200mg/kg b.w. of OG (x400). T = tubule; G = glomerulus.**
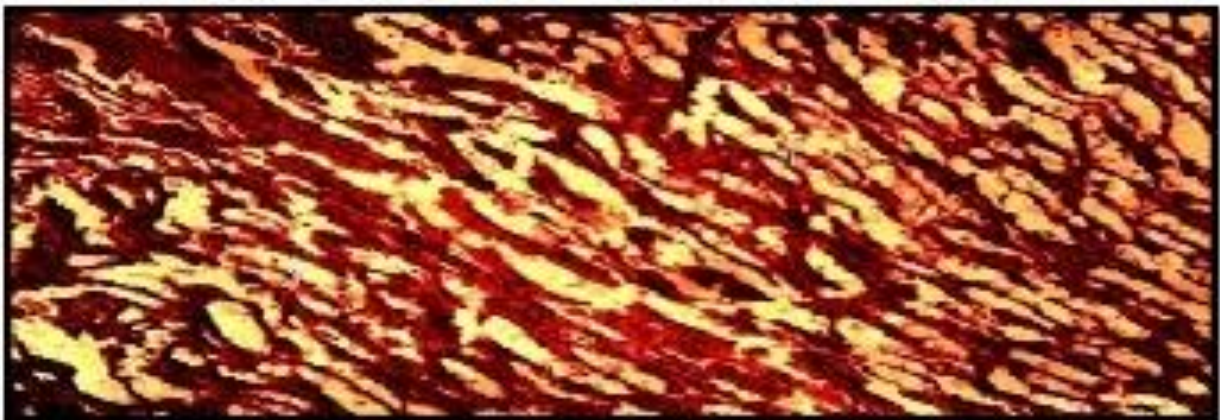


**Figure 5. Photomicrographs of kidney of diabetic rats treated with combined extract of GL and OG (GLOG) (100mg/kg b.w. of each) (x400). T = tubule; G = glomerulus; I = interstitium.**

**Figure 6. Photomicrographs of kidney of diabetic rats treated with insulin (x400). T= tubule, G= glomerulus, I= interstitium**

The changes at the molecular level impart on the gross architecture of the kidney tissues. The abnormal features observed in the kidney in untreated diabetic rats were reversed following extracts treatments. Alteration and disintegration of the glomeruli of kidney as a consequence of free radicals generated by thermoxidized lipids had been reported [9]. In this study, it is believed that these pathological changes are induced by oxidative stress associated with diabetes, and it is not unlikely that these changes precipitated by STZ-induced free radicals led to disruption in electrolyte balance as well as increased serum urea creatinine, BUN and uric acid concentrations in this study. The kidney tissue experienced some healing and regeneration with oral gavaging with the extracts of GL and OG singly and in combination through the reversal of the impressed factor responsible for oxidative stress- hyperglycaemia. Our previous study demonstrated the presence of phyto-chemicals and micronutrients with known antioxidant properties in the two leaves extract [35]. The action of these phyto-chemicals and nutrients might have arrested the free radical generation process or mop up the circulating radicals responsible for lesion and complications of diabetes, thereby reversing the pathologic changes observed in the kidney tissues of diabetic control rats.

**Conclusion**

The restoration of STZ-induced perturbation of electrolytes, the significant (p<0.05) low serum concentrations of glucose, creatinine, urea, BUN and uric acid and the kidney lesions salvaging effects observed in combined extracts treated rats relative to single extracts treated counterparts reveal a positive synergistic activity of the leaves extract. Therefore, nephroprotection against streptozotocin diabetes is more effective in combined than single leaves extract of *Gongronema latifolium* and *Ocimum gratissimum L.*

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