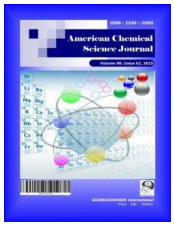

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Renal Protective Effect of Ethanolic Leaf Extract of *Gongronema latifolium* Benth in Acetaminophen-induced Renal Toxicity in Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author CI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors CI and FOU managed the analyses of the study. Authors CI and FOU managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the renal protective effect of ethanolic leaf extract of *Gongronema latifolium* Benth in acetaminophen-induced renal toxicity in albino rats.
Place and Duration of Study: Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria, between October 2014 and January 2015.
Methodology: Fifty male albino rats aged 7 weeks were used in this study. The animals were randomly placed into five (5) groups with ten (10) rats in each group. The administration of the leaf extract and acetaminophen were made through oral intubation. The animals were sacrificed, the biochemical parameters and histological analysis of the kidney accessed.
Results: Creatinine, serum urea and electrolytes: sodium and potassium increased significantly (compared with control) after the administration of acetaminophen only, but reduced significantly ($p \leq 0.05$) in all groups administered both the leaf extract and acetaminophen, except potassium which reduced non-significantly (from 12.33±0.45 to 11.94±0.56 mEq/L) in the group administered 200 mg/kg of the leaf extract (group 3) when compared with the negative control. Creatinine, serum urea, sodium and potassium reduced from 1.43±0.07 to 1.10±0.07 mg/dl, 40.65±1.57 to 28.22±1.39

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mg/dl, 145.33±2.88 to 139.55±0.93 mEq/L and 12.33±0.45 to 9.72±0.40 mEq/L respectively in the group administered 600 mg/kg of leaf extract. Chloride decreased significantly after the administration of acetaminophen only (compared with control), but increased significantly ($p \leq 0.05$) in all groups administered both the leaf extract and acetaminophen (when compared with the negative control). Chloride increased from 107.07±0.68 to 111.64±0.49 mEq/L in the group administered 600 mg/kg of leaf extract. The histological analysis of the Kidney section of rat treated with 1000 mg/kg b.wt of Acetaminophen (APAP) only show shrunken glomeruli and marked necrosis and sloughing of collecting ducts, but administration of the extract and APAP showed that the extract had a protective effect when compared with the effected kidney.

Conclusion: These results indicate that the ethanolic leaf extract of *Gongronema latifolium* Benth has protective effect on the kidney and can be used against some renal inflammations.

Keywords: Kidney; acetaminophen; Gongronema latifolium; diseases; renal impairment.

1. INTRODUCTION

Acetaminophen or paracetamol is non-narcotic analgesic and antipyretic [1]. It is as potent as aspirin especially in the central nervous system. Acetaminophen is normally well tolerated; side effects and interactions with other drugs are rare in a normal dosage. Over dosage and prolonged use of acetaminophen can cause liver impairment [2] and induction of oxidative stress. When reactive oxygen species generation exceeds the antioxidant capacity of cells, oxidative stress develops, potentially causing tissue damage [3], lipid peroxidation, plasma membrane alterations, and inactivation of enzymes [4]. The overdose of acetaminophen due to prescription is not common, though the case of acquisition of acetaminophen over-the-counter for self-medication without prescription may lead to damage to organs.

Gongronema latifolium Benth is a perennial edible plant with soft and pliable stem. It is widely used in the West African sub-region for a number of medicinal and nutritional purposes. It belongs to the family of asclepiadaceae. *Gongronema latifolium* Benth (whose leaves are bitter) is commonly called ‘‘utazi’’ and ‘‘arokeke’’ in South Eastern and South Western parts of Nigeria respectively. It is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine [5,6,7].

According to Nwanjo et al. [8], phytochemical studies of *Gongronema latifolium* leaves show the presence of Glycosides, Alkaloids, Saponin, Tannin and Flavonoids. Egbung et al. [9] reported the presence of phytochemicals (tannins, saponins, alkaloids, flavonoids and hydrocyanide), mineral elements (Cr, Cu, Se, Zn and Fe) and vitamins (A, C, riboflavin, niacin and thiamine) in the root, bark and twig extracts of *Gongronema latifolium*. However, the concentration of these phytochemicals varies among these plant parts. Tiwari and Rao [10] reported that the different composition of the active components in plants give medicinal plants an edge as better therapeutic agents than chemotherapy in management of different ailments such as atherosclerosis, hypertension and diabetes.

A cold infusion of the pounded stem of *Gongronema latifolium* is used to manage colic and intestinal symptoms usually associated with worms. The leaves are used to prepare food for nursing mothers, where it is believed to stimulate appetite, reduce post-partum contraction and enhance the return of the menstrual cycle. *Gongronema latifolium* possesses hypoglycemic activity, hypotensive, hepatoprotective and hypolipidemic effects [11,6,12]. Imo et al. [13] reported that *Gongronema latifolium* methanolic leaf extract exhibited biochemical and histological changes in acetaminophen-induced hepatic toxicity in albino rats and can be used against some hepatic inflammations.

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). In renal disease, serum creatinine values do not increase significantly until renal function has been considerably impaired. Determination of creatinine clearance ratios, however, may more sensitively indicate renal impairment. The liver produces urea in the urea cycle as a waste product of the digestion of protein.

Some of the causes of an increase in BUN include: high protein diet, decrease in Glomerular Filtration Rate (GFR) (suggestive of renal failure) and in blood volume, congestive heart failure,

gastrointestinal hemorrhage, fever and increased catabolism. The main causes of a decrease in BUN are severe liver disease, anabolic state, and syndrome of inappropriate antidiuretic hormone.

A blood test that measures the main electrolytes in the body—sodium, potassium, chloride and bicarbonate (CO_2) can be used to evaluate symptoms of heart disease and monitor the effectiveness of treatments for high blood pressure, heart failure and liver and kidney disease. The balance of sodium, potassium, chloride and bicarbonate in the blood is a good indicator of how well the kidneys and heart are functioning.

2. MATERIALS AND METHODS

2.1 Drug

Acetaminophen was purchased from a standard pharmacy shop (Ndukwe Family Chemist Nig. Ltd.) in Umuahia, Abia State, Nigeria.

2.2 Plant Materials and Extraction

The leaves of *Gongronema latifolium* Benth was harvested at Itaja-Amaegbu, Olokoro, Umuahia, Abia State, Nigeria. The plant was identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu. The plant material was sun-dried. The dried leaves of *Gongronema latifolium* Benth was milled to a powder. About 250 g of the powder was extracted with 625 ml of ethanol by cold maceration for 48 hours and filtered. The filtrate was evaporated to dryness and the ethanol recovered. The concentration of the extract was made in normal saline for the experiment (200 mg, 400 mg and 600 mg).

2.3 Experimental Animals

Fifty male albino rats aged 7 weeks were used in this study. The rats were bought and kept in the animal house, Department of Biochemistry, Faculty of Biological and Physical Science, Abia State University, Uturu. The animals were allowed to acclimatize for 7 days under standard laboratory conditions with free access to commercial rat feed and water.

2.4 Experimental Design

The animals were randomly placed into five (5) groups with ten (10) rats in each group. Group 1 served as the control group (it received a placebo of normal saline). Group 2 received acetaminophen (1000 mg/kg b.w.) only: as negative control. Group 3 received 200 mg/kg of leaf extract of *G. latifolium* Benth and acetaminophen (1000 mg/kg b.w.). Group 4 received 400 mg/kg of leaf extract of *G. latifolium* Benth and acetaminophen (1000 mg/kg b.w.). Group 5 received 600 mg/kg of leaf extract of *G. latifolium* Benth and acetaminophen (1000 mg/kg b.w.).

Groups 2, 3, 4 and 5 received the acetaminophen every 24 hours for twenty one (21) consecutive days. One hour before the daily administration of acetaminophen, the test animals (groups 3, 4 and 5) received the leaf extract as stated above.

In the test groups, the drug and extract were administered through oral route using a gavage tube. All animals were allowed free access to food and water *ad libitum*.

2.5 Blood Collection

On the twenty second day, the animals were fasted, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture from each animal into dry test tubes. The blood sample was allowed to stand for about 15 minutes to clot and further spun in a centrifuge. Serum was separated from the clot with Pasteur pipette into sterile sample test tubes for the measurement of kidney function.

2.6 Biochemical Analysis

The serum concentrations of Creatinine and Urea were determined using auto-analizer (Biosystem A25 Random Access Analyzer). Serum concentrations of Sodium, Potassium and Chloride were determined using auto-analizer (EasyLyte Plus Analyzer).

2.7 Histological Analysis

After sacrificing the animals, the kidney of representatives of each of the five groups were taken for histological analysis.

2.8 Statistical Analysis

The results were subjected to statistical analysis using Analysis of Variance (ANOVA) and standard student-T-distribution test. Group means were compared for significance at p≤0.05. Data were represented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The kidney helps in maintaining homeostasis of the body by reabsorbing important materials and excreting waste products. Creatinine is a break down waste product formed in the muscle by creatine phosphate metabolism. Creatine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle for energy production. Creatinine retention in the blood is evidence of kidney impairment [14]. Creatinine retention was observed by a significant increase in creatinine level in acetaminophen-induced renal toxic group (group 2) when compared with the normal control. Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of urea cycle, where ammonia is converted into urea and excreted through urine. It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion [14]. Renal diseases which diminish the glomerular filtration lead to urea retention. Administration of acetaminophen (only) causes nephrotoxicity as indicated by significant (P≤0.05) elevation in serum level of creatinine and urea (Table 1). In this study, it is evident that elevation in urea and creatinine levels can be attributed to the damage of nephron structural integrity. The different doses of ethanolic leaf extract of *Gongronema latifolium* Benth significantly (P≤0.05) lowered urea and creatinine levels when compared with the acetaminophen-induced group (group 2), thereby stabilizing these parameters. This indicates that the ethanolic leaf extract of *G. latifolium* Benth can protect renal function against some kidney disease in albino rats.

The stability of the concentrations of sodium, potassium and chloride in the blood is a good indicator of effective functioning of the kidneys and heart. Sodium is associated with blood pressure and in many hypertensive patients, a reduction in sodium intake lowers blood pressure. Sodium increased in the group administered acetaminophen only (compared with the normal control), but decreased significantly (p≤0.05) in all groups administered both the leaf extract and acetaminophen (compared with the negative control). On the other hand, potassium, which is in the intra-cellular fluid, has been reported to be among the protective electrolytes against hypertension [15]. The decrease in the serum K^+ (in the groups administered the leaf extract) when compared with group two is an indication that membrane channels may possibly be protected or stabilized by *Gongronema latifolium* Benth leaf extract. There were significant increase in sodium and potassium levels and a decrease in chloride level in acetaminophen-induced renal toxicity group. The administration of ethanolic leaf extract of *Gongronema latifolium* Benth protected these alterations (Table 1). Significant alteration in the concentration of these body electrolytes is indicative of poor renal functions or renal impairment.

Table 1. Kidney function parameter concentrations

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Creatinine (mg/dl)	1.13±0.08	1.43±0.07 [a]	1.26±0.07 [b]	1.12±0.15 [c]	1.10±0.07 [d]
Serum urea (mg/dl)	27.77±0.87	40.65±1.57 [a]	34.91±1.26 [b]	32.85±2.54 [c]	28.22±1.39 [d]
Sodium (mEq/L)	139.72±0.83	145.33±2.88[a]	140.59±0.99 [b]	140.23±1.45 [c]	139.55±0.93 [d]
Potassium (mEq/L)	9.45±0.57	12.33±0.45 [a]	11.94±0.56 [a]	10.67±0.45 [b]	9.72±0.40 [c]
Chloride (mEq/L)	112.91±1.62	107.07±0.68[a]	110.35±1.21 [b]	111.47±0.39 [c]	111.64±0.49 [d]

Results represent mean ± standard deviation of group serum results obtained (n=10)
Mean in the same row, having different alphabet are statistically significant (p≤0.05) compared with the negative control (group two)

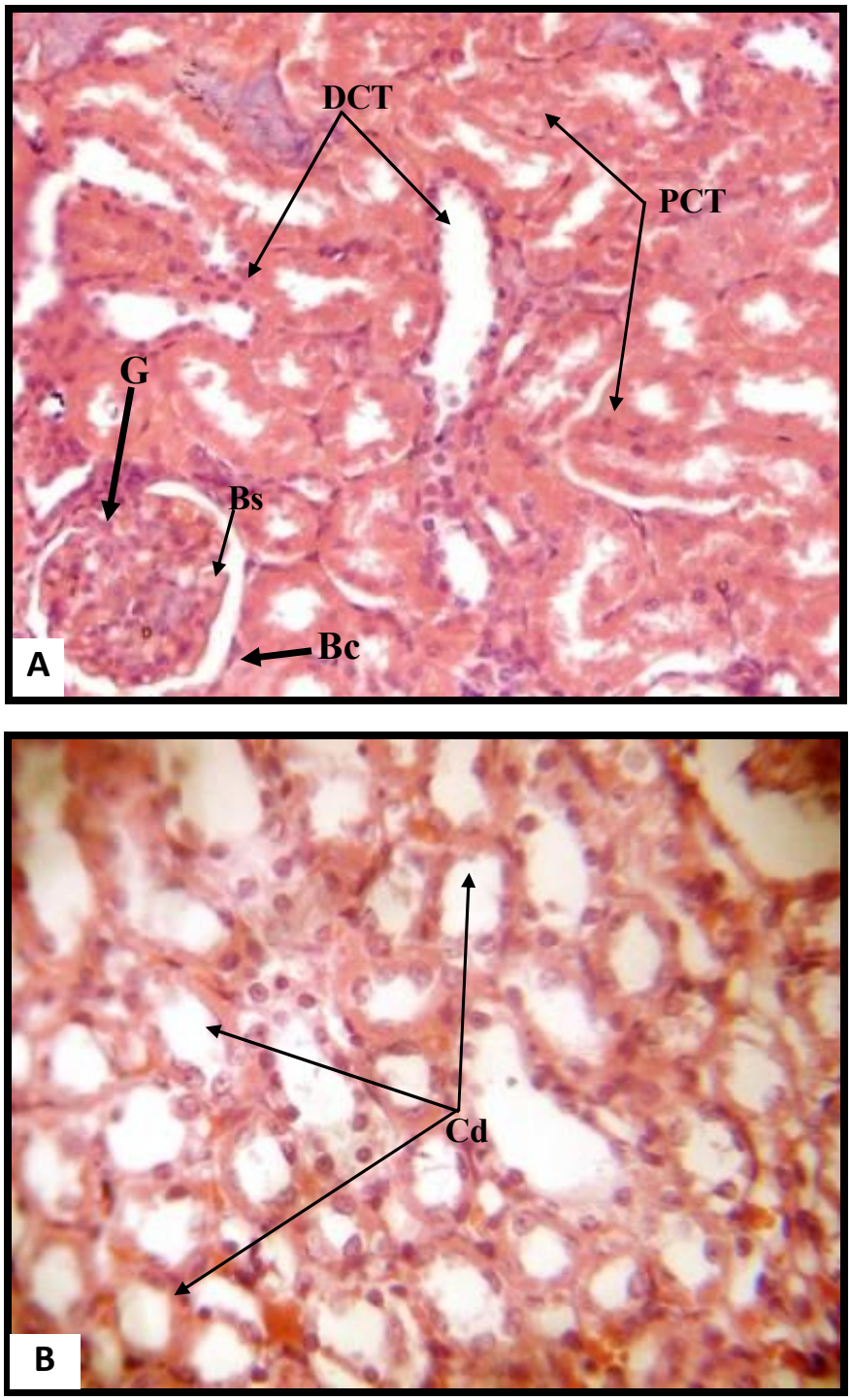

Plate 1. Photomicrographs of sections of Kidney cortex [plate 1A] and medulla [plate 1B] from rat in group one [normal control] showing the normal histoarchitecture of the renal tissue. The intact glomerulus [G], Bowman's capsule [Bc] and space [Bs], proximal and distal convoluted tubules [PCT and DCT] in the cortex and the medullary collecting ducts [Cd] are shown.
[STAIN: H & E] [Mag: A – x400; B – x400]

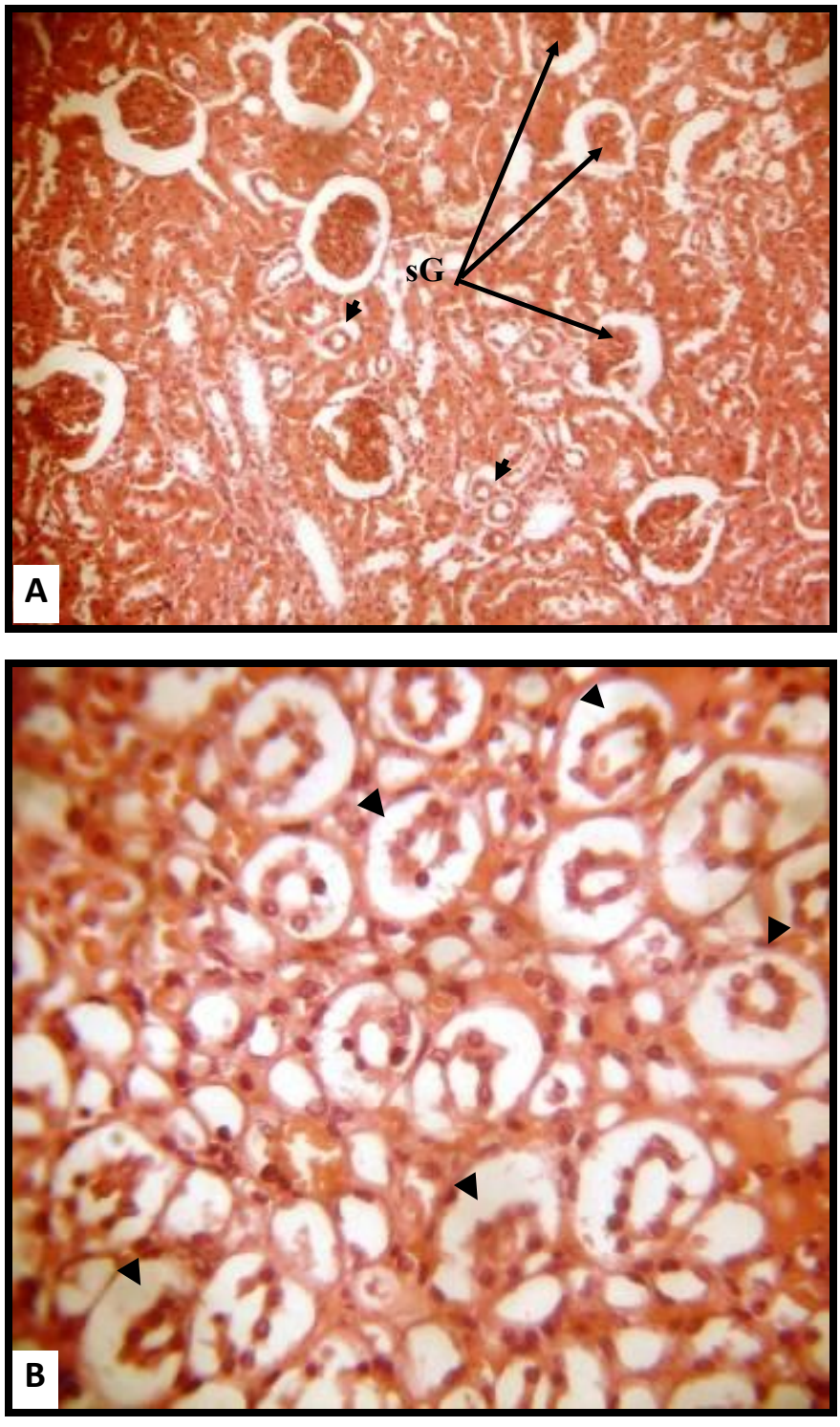

Plate 2. Photomicrographs of sections of Kidney cortex [plate 2A] and medulla [plate 2B] from rat in Group two treated with 1000 mg/kg b.wt of acetaminophen only. Histopathological changes observed are shrunken glomeruli [sG] and marked necrosis and sloughing of collecting ducts [arrowheads]

[STAIN: H & E] [Mag: A – x400; B – x400]

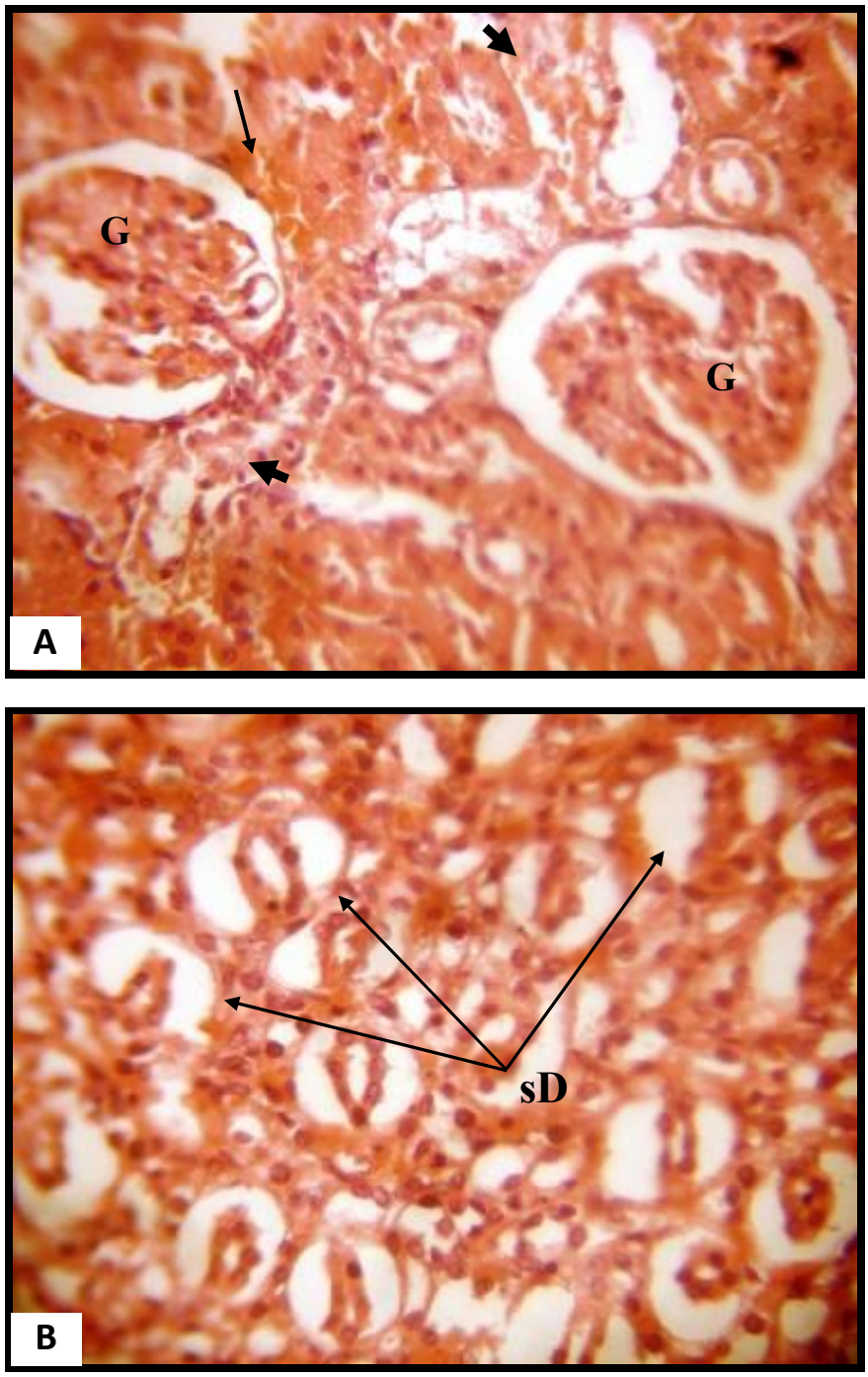

Plate 3. Photomicrographs of sections of Kidney cortex [plate 3A] and medulla [plate 3B] from rat in group three administered 200 mg/kg b.wt. of the leaf extract and acetaminophen. The glomeruli appear mildly shrunken [G]. However, degeneration of some tubules [thick arrows] and sloughed ducts [sD] are evident
[STAIN: H & E] [Mag: A- x400; B – x400]

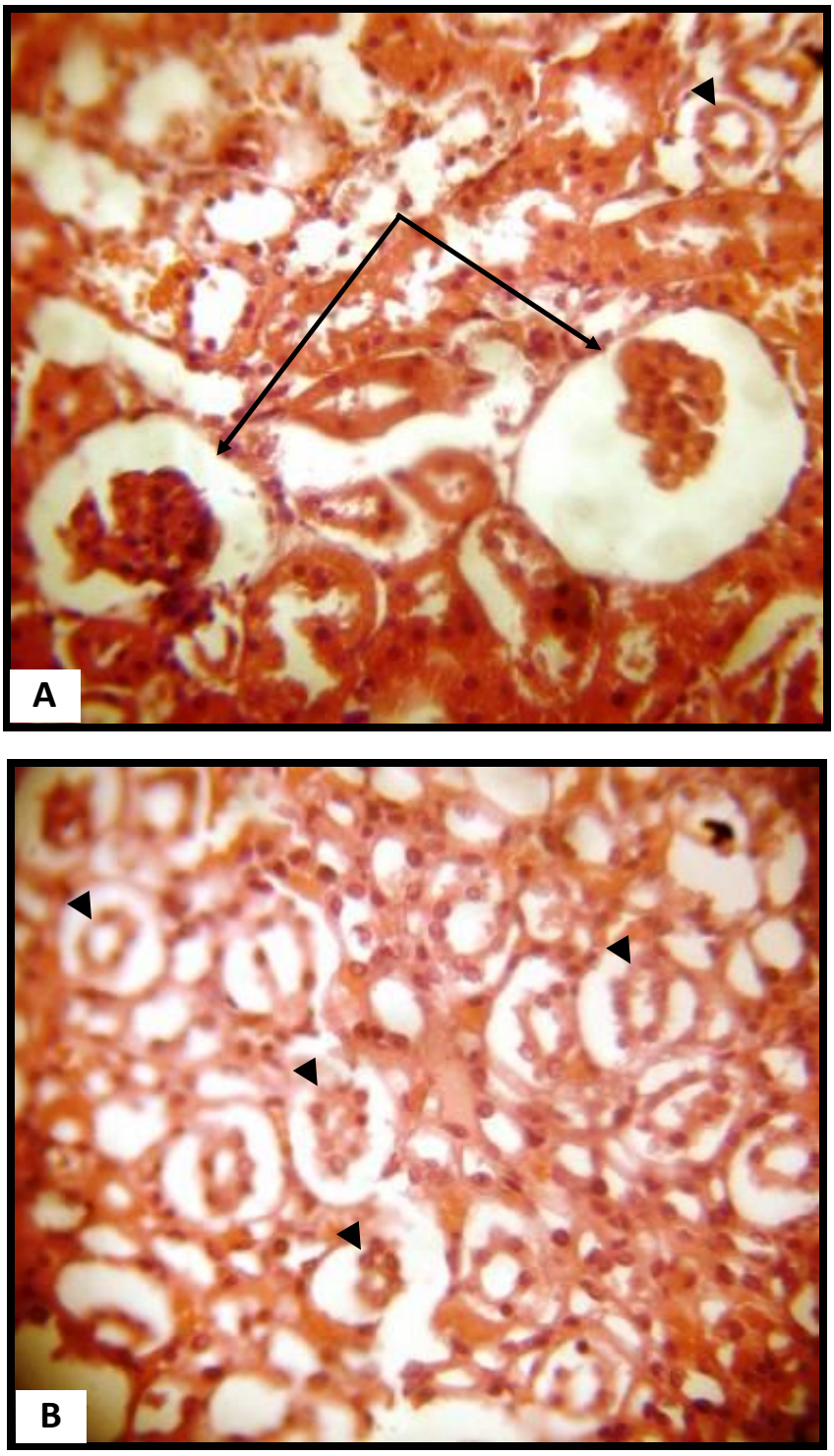

Plate 4. Photomicrographs of sections of Kidney cortex [plate 4A] and medulla [plate 4B] from rat in group four administered 400 mg/kg b.wt. of the leaf extract and acetaminophen. There is marked glomerular shrinking [arrows] and collecting ducts appear necrotic and sloughed [arrowheads]

[STAIN: H & E] [Mag: A – x400; B – x400]

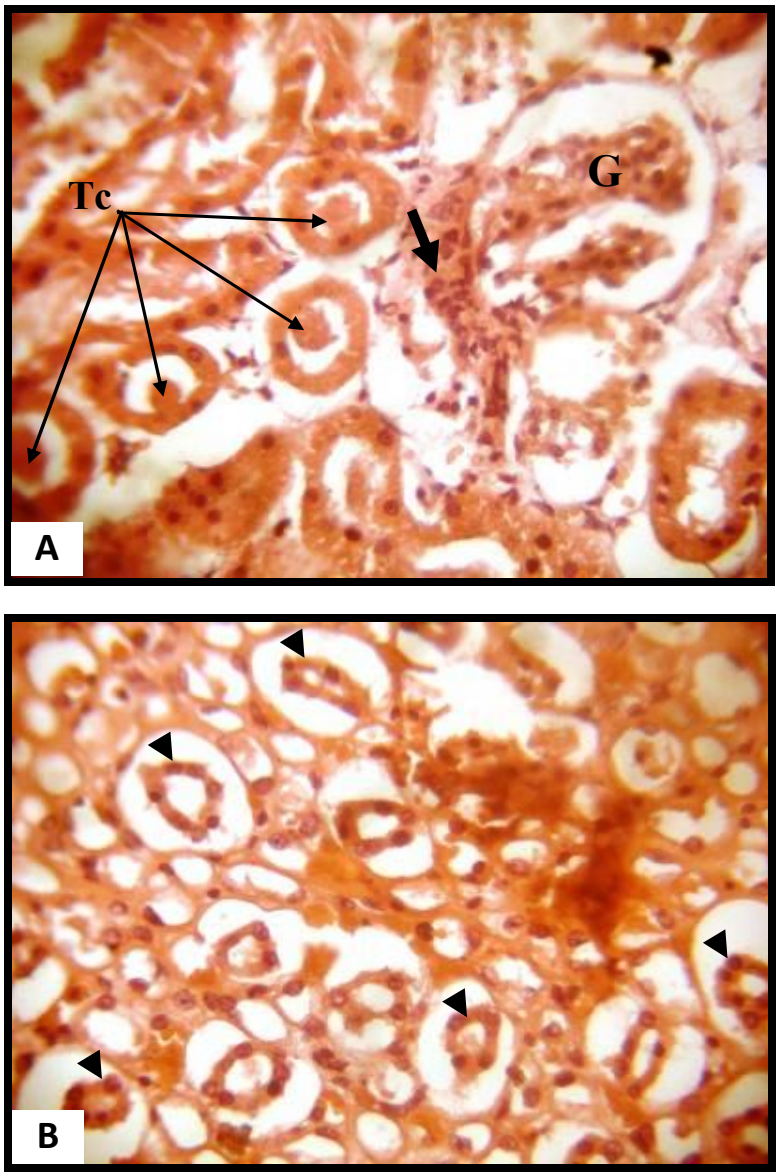

Plate 5. Photomicrographs of sections of Kidney cortex [plate 5A] and medulla [plate 5B] from rat in group five administered 600 mg/kg b.wt. of the leaf extract and acetaminophen showing degenerating glomeruli [G] and tubules with cellular infiltrates [thick arrow], eosinophilic tubular casts [Tc] and markedly sloughed degenerating ducts [arrow heads]
[STAIN: H & E] [Mag: A – x400; B – x400]

4. CONCLUSION

The results of this study indicate that ethanolic leaf extract of *Gongronema latifolium* Benth has a protective effect against acetaminophen-induced renal toxicity and can be used against renal inflammations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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