

Effect of ethanol stem extract of *homalium letestui* on gentamicin-induced kidney Injury in rat

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ABSTRACT

Background and aims: The kidney is a very vital organ that is responsible for regular elimination of unwanted substances from the body. It is frequently exposed to conditions that expose it to damage. There are not enough drugs in the market to protect or treat the kidney against these deleterious conditions. *Homalium letestui* has been used in traditional medicine as an antidote. This study aims to investigate the nephroprotective activity of ethanol stem extract of *Homalium letestui* against gentamicin-induced rat's kidney injury.

Methods: Thirty-six (36) adult male albino rats were used for this study. Animals in group I served as the control with normal saline, group II received gentamicin (100 mg/kg), group III received Silymarin (100 mg/kg), while group IV, V and VI were administered 250, 500 and 750 mg/kg of ethanol stem extract of *Homalium letestui* along with gentamicin 100 mg/kg for 8 days. The nephroprotective effect of the extract was evaluated by the assay of kidney function parameters such as serum urea, creatinine, electrolytes (K⁺, Na⁺, Cl⁻ and HCO⁻³) and histopathological examination of the kidney.

Results: *Homalium letestui* caused significant $p \leq 0.05$ dose-dependent reduction in the level of serum urea, creatinine, and electrolytes in rat's kidney when compared to the control. The chemical pathological changes were consistent with histopathological observations suggesting marked the nephroprotective effect of the stem extract of *H. letestui*.

Conclusion: The result of the study suggests that the plant has kidney protective ability which supports its traditional use as an antidote

Keywords: *Homalium letestui*, nephroprotective, rats

INTRODUCTION

The kidney consists of a pair bean-shaped organs that serve several essential regulatory roles in vertebrate animals¹. They remove excess organic molecules (e.g. glucose) and it is by this action that their best-known function is performed: the removal of waste products of metabolism¹. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood and remove water-soluble wastes, which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids². The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme renin, the latter of which indirectly acts on the kidney in negative feedback¹. The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood

pressure³. Nephrotoxins are chemicals displaying nephrotoxicity. The nephrotoxic effect of most drugs is more profound in patients already suffering from renal impairment. Some drugs may affect renal function in more than one way⁴.

Homalium letestui Pellegr (Flacourtiaceae) is a tree with a long straight, slender bole attaining about 27 m height, occasionally up to 33 m, and to 1 m girths. It is locally known by the Ibibio's as Otong idim, akpurukwu by the Igbos and abo àkó by the Yoruba people of Nigeria. Bark sap is applied as enema and bark pulp rubbed in to treat oedema. Bark decoctions are taken in mixtures to treat orchitis and as a tonic for women after childbirth. Root extracts are administered to treat malaria. The tree is decorative with its showy flowers, fruits, and reddish young leaves, and is sometimes planted as ornamental⁵. In Ivory Coast sap from the bark is used in enemas for the treatment of generalized oedemas while lees from the bark are rubbed over the area⁶. In Gabon, a bark-decoction with other drug-plants is taken by draught for orchitis, and bark-scrapings enter a prescription given to a newly-delivered

woman .The Yoruba of Nigeria calls on the plant in an incantation against small-pox, while the bark, finely ground to a powder, is blown by Liberian witchdoctors into a dragon's lair to stupefy it before slaying it⁷.

Several research work has been done on pharmacological activities of the plant parts. These include antiulcer⁸, antiarrheal⁸ antiplasmodial⁹, antidiabetic¹⁰, anti-inflammatory and analgesic¹¹, cellular antioxidant, anticancer, and antileishmanial¹¹, depressant and anticonvulsant¹² antibacterial¹³ and *in vitro* antioxidant activity against DPPH¹³. The aim of this study is to investigate the nephroprotective ability of ethanol stem extract of *Homalium letestui* on gentamicin-induced kidney rat's injury.

METHODS

Plants collection

Homalium letestui (stem) was collected from a forest in Uruan area, Akwa Ibom State, Nigeria. It was identified and authenticated by Dr. Margaret Basse of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPUU 382) was deposited at Department of

Pharmacognosy and Natural Medicine Herbarium.

Extraction

The stem was washed and dried under shed for two weeks. The dried plant material was cut into smaller pieces and grounded to powder. The powdered material was macerated in 70% ethanol. The liquid filtrate was evaporated to dryness *in vacuum* 40°C using rotary evaporator. The ethanol extract was stored at -4°C until used.

Animals

Adult male albino rats were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

Animal treatment

36 rats were weighed and divided into six groups with 6 animals per group. Treatment was as follows: Group 1 consisted of normal animals that were administered with normal saline (10 ml/kg) for eight days, Group 2, the received gentamicin 100 mg/kg for eight days. Group 3 served as the

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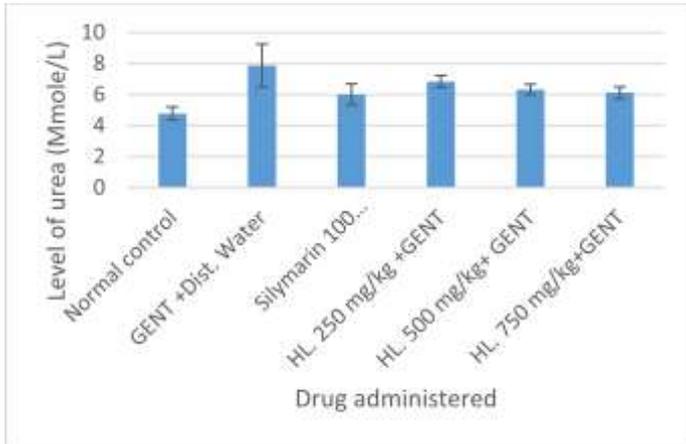


Figure 1: Effect of *H. letestui* level of serum urea

standard group and rats in this group were administered 100 mg/kg body weight of silymarin orally for 8 days, while groups 4, 5 and 6 were administered p.o with gentamicin 100 mg/kg concomitantly with 250, 500 and 750 mg/kg of *H. letestui* stem extract respectively daily for 8 days. On the 8th day, animals were weighed again and sacrificed under light diethyl ether vapor.

Determination of the protective effect of the extract on biochemical parameters and histology of kidney of rats

The various serum samples collected after treatment of the animals were analyzed according to standard methods for the effect of the extract on various biochemical parameters of rats such as urea, creatinine as well as some ions

like sodium, potassium, and chloride. This analysis was done at Department of Chemical Pathology, University of Uyo Teaching Hospital, (UUTH), Uyo using various diagnostic kits like Randox Laboratory kits, Dialab diagnostic kits, HUMAN diagnostic kits and TECO analytical kits. The kidneys of the animals fixed in 10% formaldehyde were processed, sectioned and stained with Haematoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo.

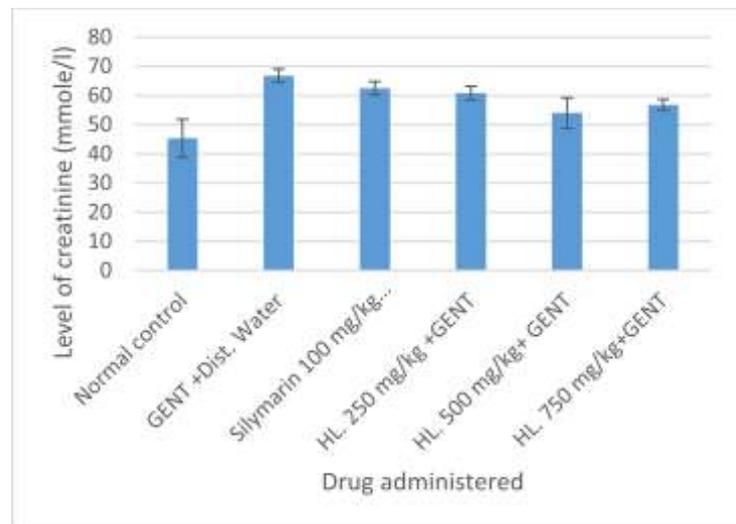


Figure 2: Effect of *H. letestui* on level of creatinine

Kidney Function Test

The following biochemical parameters were assayed as markers of kidney function using diagnostic kits; Level of

electrolytes (Na^+ , K^+ , Cl^- , and HCO_3^-), creatinine and blood urea. The above parameters were determined at the Chemical Pathology Department of University of Uyo Teaching Hospital.

Statistical Analysis and Data Evaluation

Data obtained from this study were analyzed statistically using Student's t-test and ANOVA (One - way) followed by a post test (Tukey-Kramer multiple comparison tests). Differences between means were considered significant at 5%, 1% and 0.1% level of significance i.e $P \leq 0.05$, 0.01 and 0.001.

RESULTS

Effect of Treatment with Ethanol Stem Extract of *Homalium letestui* on the Blood Hematological parameters, in rats with Gentamicin-induced hepatotoxicity

Gentamicin treatment did not affect RBC, Hb, and WBC levels of rats treated with it. However, neutrophils percentage was significantly ($p < 0.05 - 0.01$) reduced by gentamicin treatment. Lymphocytes, eosinophils and monocytes percentages of various groups were significantly ($p < 0.01 -$

0.001) increased when compared to normal control. Platelets count which was significantly ($p < 0.05$) reduced by gentamicin treatment was significantly elevated following treatment with the stem extract and silymarin (Table 1).

Nephroprotective Effect of *Homalium letestui* Stem Extract Against Gentamicin-Induced Kidney Injury.

Pretreatment of rats with the stem extract of *H. letestui* (250 – 750 mg/kg BW) prior to treatment with gentamicin was found to protect the animals from kidney injuries. Serum creatinine and serum urea levels were found to be significantly ($p < 0.05 - 0.001$) elevated in rats treated only with gentamicin when compared to normal control; whereas treatment with the stem extract significantly ($p < 0.05 - 0.001$) lowered their levels in the treated animals dose-dependently. The reductions in the levels of serum creatinine and urea were significantly ($p < 0.01 - 0.001$) dose-dependent. Silymarin (a reference drug) also caused significant ($p < 0.01 - 0.001$) reductions in the levels of serum creatinine and urea compared to the control. However, the level sodium ion significantly ($p < 0.005 - 0.001$)

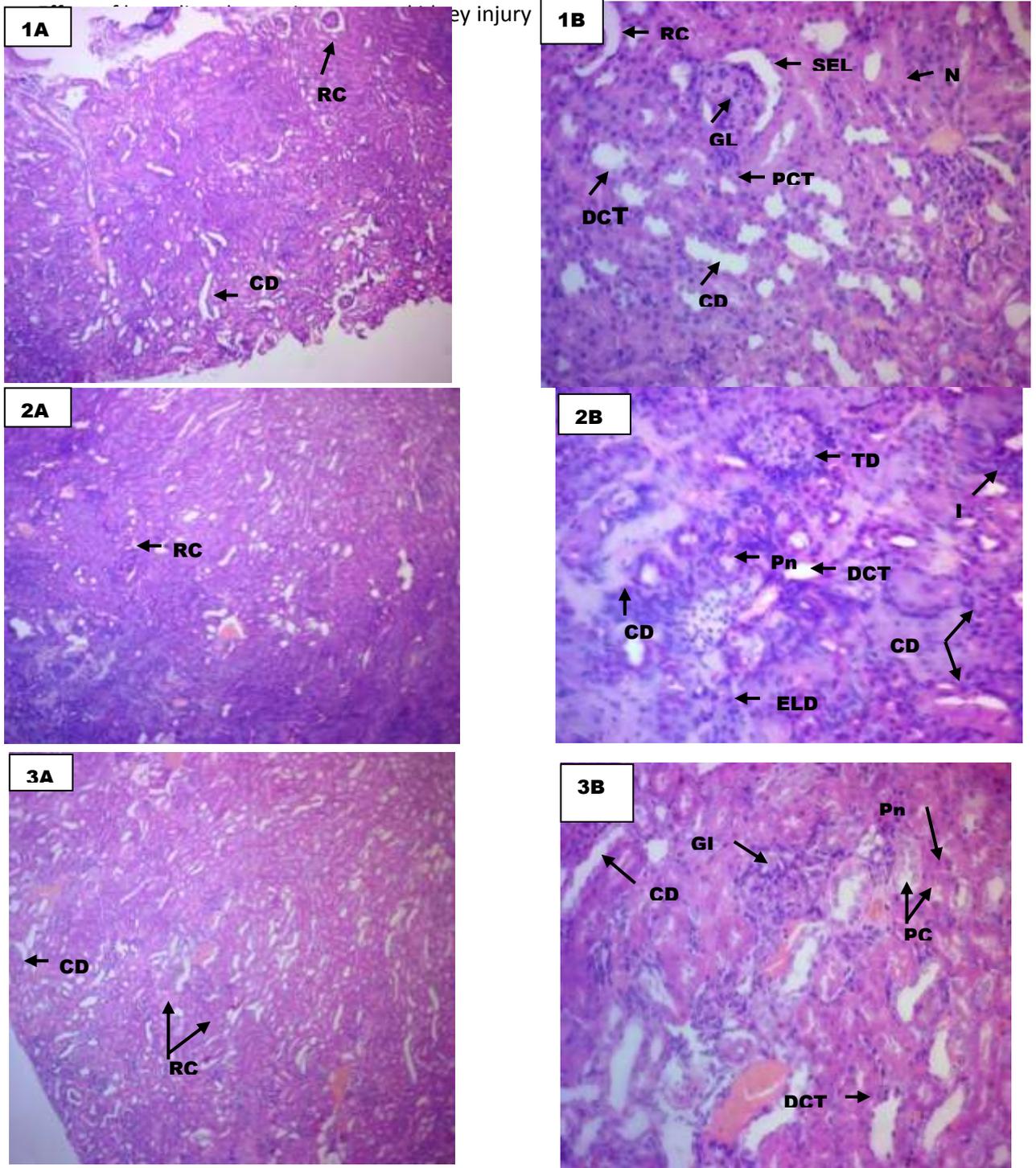


Figure 3: Histological sections of Kidneys of rats treated with *Homalium letestui* 250 mg/kg bw and Gentamicin (4), *Homalium letestui* 500 mg/kg bw and Gentamicin 100 mg/kg bw (5) and *Homalium letestui* 750 mg/kg bw and Gentamicin 100 mg/kg bw (6) at magnification A (x100) and B(x400) stained with H&E Technique.

Keys: Renal corpuscle (RC) Convoluted tubules (CT), Tubular necrosis (TN), Tubular degeneration (TD), Collecting ducts (CD), Degenerating collecting duct (DCT), Epithelial lining (ELD), Cellular proliferation (Cp), Inflammation (I) and Pyknotic nucleus (Pn)

increased following gentamicin treatment when compared to normal control (Table 2). These increases were reversed significantly ($p < 0.01$) and dose-dependently by pretreatment with the stem extract.

DISCUSSION

Gentamicin is a nephrotoxin because a small but sizable proportion of the administered dose is usually retained in the epithelial cells of the proximal tubules after glomerular filtration¹⁴. This induces noticeable and characteristic changes in lysosomes of proximal tubular cells consistent with the accumulation of polar lipids (myeloid bodies). These changes are preceded and accompanied by signs of tubular dysfunctions or alterations (release of brush-border and lysosomal enzymes; decreased reabsorption of filtered proteins; wasting of K^+ , Mg^{2+} , Ca^{2+} , and glucose; phospholipiduria; and cast excretion¹⁵. Gentamicin also induces oxidative stress through induction of reactive oxygen species such as free radical, superoxide, hydroxyl radical anion and hydrogen peroxide¹⁶. When ROS are generated as

a consequence to tissue injury induced by gentamicin, there is an attack on different cell components such as DNA, RNA, proteins, lipids and enzymes leading to many degenerative processes in the renal cells manifested as glomerular disease, renal ischemia, perfusion injury and eventually acute renal failure¹⁶. The effect of ROS in the body is usually suppressed by antioxidant enzyme systems. Gentamicin-induced kidney injury is presented by an increase in serum levels of creatinine, urea, uric acid as well as severe proximal renal tubular necrosis followed by renal failure¹⁷. Total leukocyte count was increased in gentamicin-treated animals. Blood indices RBC, PCV, WBC and Hb did not vary significantly. Gentamicin caused an increase in the level of monocytes, basophils, eosinophils and lymphocyte which appears to be reversed in the groups pretreated with the extract. Gentamicin also caused a decrease in the platelet count, an effect that was improved by the plant extract. The effect of the extract on the platelet level of the rats appeared to be biphasic, that is, the effect was optimal at a

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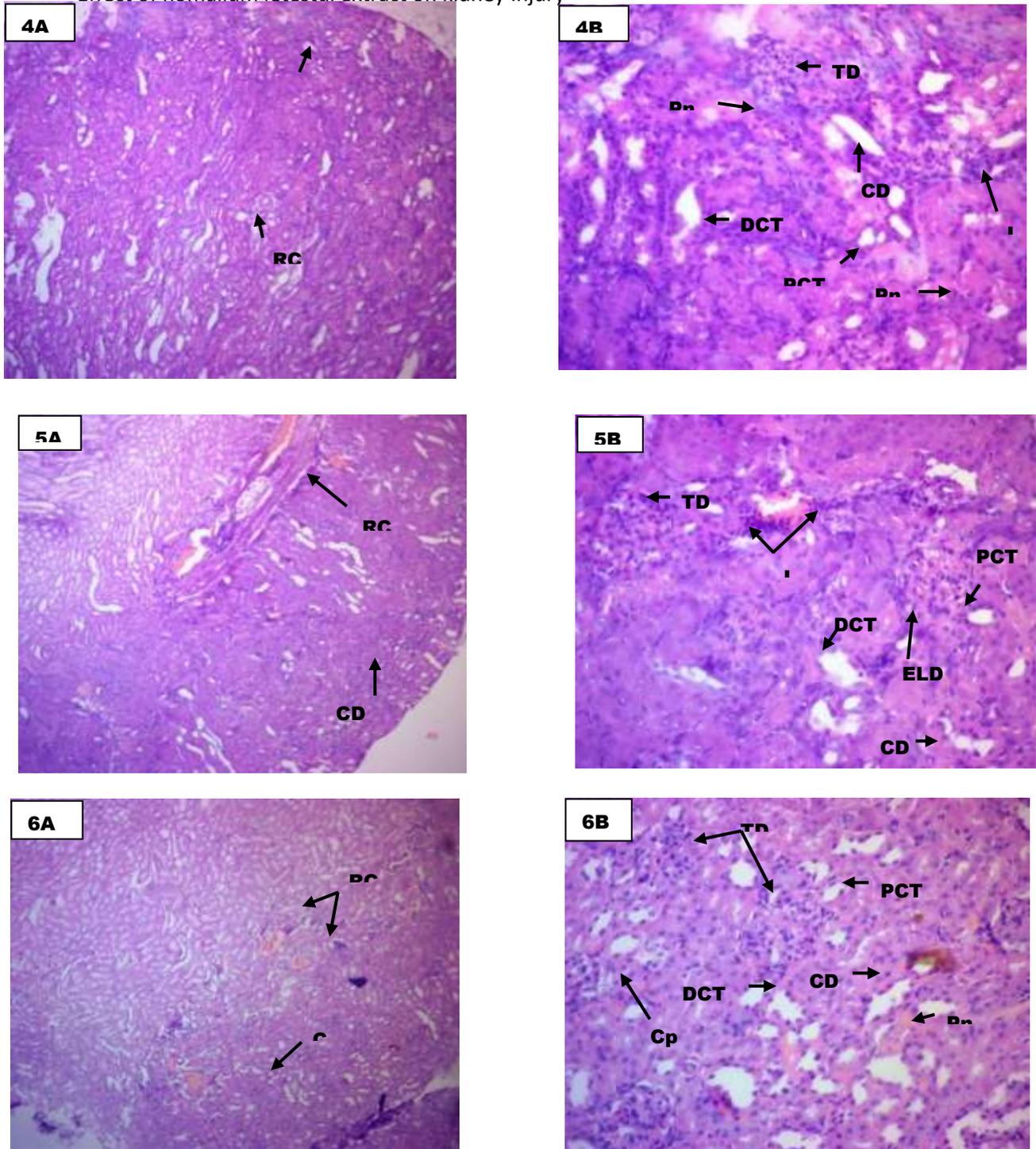


Figure 4: Histological sections of Kidneys of rats treated with *Homalium letestui* 250 mg/kg bw and Gentamicin (4), *Homalium letestui* 500 mg/kg bw and Gentamicin 100 mg/kg bw (5) and *Homalium letestui* 750 mg/kg bw and Gentamicin 100 mg/kg bw (6) at magnification A (x100) and B(x400) stained with H&E Technique.

Keys: Renal corpuscle (RC) Convoluted tubules (CT), Tubular necrosis (TN), Tubular degeneration (TD), Collecting ducts (CD), Degenerating collecting duct (DCT), Epithelial lining (ELD), Cellular proliferation (Cp), Inflammation (I) and Pyknotic nucleus (Pn)

Particular dose beyond which the effect apparently reversed. This may be due to the chemical components of the plant or partial agonist effect.

Increase in the level of serum creatinine is indicative of glomerular filtration rate reduction which is often associated with increases in serum urea and uric acid as was seen in the study.

The administration of stem extract of *Homalium letestui* produced prominent decreases in serum levels of creatinine and urea-induced by gentamicin. Also, the stem extract pretreatment significantly ($p < 0.01 - 0.001$) and dose-dependently reduced the elevated levels of some ions like sodium, potassium and chloride which were increased following gentamicin treatment when compared to normal control. The suppression of gentamicin-induced nephrotoxicity by the extract may have resulted from the antioxidant and free radical scavenging potentials of the extract. Histopathological examination agrees with biochemical result suggesting that the plant has nephroprotective activity. The extract significantly and dose-dependently protected the basement membrane of

kidney with intact membrane architecture at the high dose administered. This effect may be due to the antioxidant activity of the plant extract. Literature has shown that medicinal plants with nephroprotective properties mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids, phenol and other active compounds¹⁸. This is in agreement with the findings of this study. It has been reported that the plant has antioxidant constituent^{13,19}. The phenolic and flavonoid component may be responsible for this effect. Flavonoids, tannins, and phenols have been reported to exert profound *in vitro* and *in vivo* stabilizing effect on the lysosomes of experimental animals²⁰. Plant flavonoids which show antioxidant activity *in vitro* also function as antioxidants *in vivo*²¹. Phenolic compounds function as high-level antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species. Again, a strong relationship between the total phenolic content and antioxidant activity in

fruits, vegetables, grain products, and plant subjects of ethnopharmacological treatments has also been reported²². The nephroprotective activity of this extract maybe through the antioxidant activity of the plant as reported by Ita and Ngochindo¹³ and Okokon, Joseph and Emem¹⁹.

CONCLUSIONS

The results from biochemical parameters and the histological study show that the plant possesses chemical constituent that has a nephroprotective activity which justifies its use traditionally as an antidote.

CONFLICT OF INTEREST

All authors disclose any financial and personal relationships with other people or organizations and the authors declare that there are not any potential conflicts of interest.

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Table 1: Effect of ethanol stem extract of *Homalium letestui* on the hematological parameters of rats with Gentamicin-induced nephrotoxicity.

Parameters Treatment Dose (mg/kg)	RBC (X 10 ¹² /l)	PCV (%)	Hb (g/dl)	WBC (X 10 ⁹ /l)	Neutrophils. (%)	Lymphocy tes (%)	Monocyte s (%)	Eosinophils (%)	Basophils (%)	Platelet (%)
Normal control	4.66±0.12	48.66±0.9	16.08±0.4	13.63±0.5	61.1±4.80	35.5±5.63	2.50±0.76	0.83±0.40	0.10±0.01	134.0±21.03
GENT +Dist. Water	4.93±0.12	51.16±1.5	17.5±0.70	14.35±0.5	54.16±6.01 ^a	38.0±5.44	5.16±1.57 ^c	2.66±0.66 ^a	0.12±0.01	120.5±17.46 ^a
Silymarin 100mg/kg +	4.81±0.2	53.33±2.8	17.76±0.8	12.93±0.2	49.50±5.05 ^b	45.0±6.05 ^a	4.83±1.35 ^b	1.66±0.01 ^d	0.01±0.01 ^f	178.0±16.11 ^{cf}
GENT HL. 250mg/kg +	3.98±0.52	49.33±1.6	16.15±0.5	13.18±0.2	46.0±5.82 ^b	46.66±6.6 ^a	5.00±1.56 ^c	3.66±0.76 ^{bd}	0.01±0.01 ^f	150.7±24.67 ^{cf}
GENT HL. 500mg/kg+	5.35±0.21	57.5±1.70	19.11±0.5	13.40±0.3	56.83±7.51 ^a	41.16±5.0 ^{8^a}	4.0±1.03 ^b	2.16±0.79 ^a	0.00±0.00 ^f	180.16±17.78 ^{cf}
GENT HL. 750mg/kg+	4.78±0.1	52.83±2.4	17.36±0.3	15.25±0.1	56.66±4.83 ^a	35.5±5.09 ^c	6.16±0.89 ^c	1.16±0.60 ^d	0.01±0.01 ^f	150.0±16.70 ^{cf}

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control. dp< 0.05, ep< 0.01, fp<

0.001 when compared to Gentamicin . n = 6

Table 2: Effect of ethanol stem extract of *Homalium letestui* on some kidney function parameters of gentamicin –induced kidney injury in rat

Parameters/ Treatment	Na ⁺ (mmol/l)	K ⁺ (mmol/l)	Cl ⁻ (mmol/l)	HCO ₃ ⁻ (mmol/l)	UREA (mmol/l)	Creatinine (mmol/l)
Normal control	65.06±3.59	6.77±0.36	66.58±0.93	15.38±0.75	4.78±0.42	45.33±6.48
GENT +Dist. Water	85.53±1.75 ^b	8.08±0.29	72.23±2.98	18.35±0.59	7.86±1.38 ^c	66.83 ±2.30 ^b
Silymarin 100 mg/kg + GENT	66.00±2.24 ^e	6.36±0.33 ^e	62.05±2.91 ^e	18.06±0.39	6.01±0.68 ^d	62.50±2.31 ^b
HL. 250 mg/kg +GENT	66.06±3.52 ^e	6.37±0.21 ^e	61.71±2.16 ^e	17.26±0.45	6.83±0.39 ^e	60.83±2.31 ^a
HL. 500 mg/kg+ GENT	68.36± 3.45 ^d	6.11±0.25 ^f	61.58±3.82 ^e	17.36±0.38	6.33±0.35 ^e	54.16±5.19 ^d
HL. 750 mg/kg+GENT	73.85± 1.58	5.75±0.15 ^f	58.01±1.85 ^f	17.16±0.23	6.13±0.38 ^e	56.83±1.83 ^f

Data were expressed as mean ± SEM. significant at ap< 0.05, bp< 0.01, cp< 0.001 when compared to control.

dp< 0.05, ep< 0.01, fp< 0.001 when compared to gentamicin group . n = 6