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Jude E Okokon
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

John A Udobang
Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Uyo, Uyo, Nigeria

Daniel N Obot
Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Uyo, Uyo, Nigeria

Eucharia C Agu
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

Corresponding Author:
John A Udobang
Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Uyo, Uyo, Nigeria

Nephroprotective activity of husk extract of *Zea mays* against gentimicin-induced kidney injury in rats

Jude E Okokon, John A Udobang, Daniel N Obot and Eucharia C Agu

Abstract
Background: *Zea mays* L. (Poaceae) is used traditionally by the Ibibios of Southern Nigeria to treat stomach ulcer, malaria, inflammatory diseases and as an antidote.
Objective: To evaluate the nephroprotective property of cornhusk extract against gentimicin-induced kidney injuries to ascertain the folkloric claim of its usefulness in the treatment of poisoning.
Methods: The husk extract of *Zea mays* (187-748 mg/kg) was investigated for nephroprotective potential against against gentimicin-induced kidney injuries in rats. Assays of kidney function parameters as well as histopathological study of the kidney were used to assess nephroprotective activity of husk extract.
Results: Administration of the husk extract (187-748 mg/kg) caused significant ($p< 0.05$) reduction of high levels of serum creatinine, urea and electrolytes concentrations (K^+, Na^+, Cl^- and HCO^-_3) caused by the toxicants. The effects were dose-dependent in most cases. Histology of the kidney sections of extract and silymarin-treated animals showed reductions in the pathological features compared to the organotoxic-treated animals. The chemical pathological changes were consistent with histopathological observations suggesting marked nephroprotective potentials.
Conclusion: The results showed that husk extract of *Zea mays* has nephroprotective potential against injurious agents which may be due to the activities of its phytochemical components.

Keywords: *Zea mays*, husk, kidney, renoprotective

1. Introduction
Zea mays L. (Poacae) also known as maize or corn, is an annual grass plant cultivated throughout Nigeria primarily for human consumption and as animal feed. The plant is tall with a fibrous root system and has long narrow leaves on opposite side of the stem and bears ears that are enclosed in modified leaves known as husks [1]. In addition to its nutritive values, various parts of the plant are also used in Ethnomedicine for the treatment of several ailments. The corn silk is used as an antidiabetic or diuretic, and decoction of the silk is consumed for the treatment of urinary troubles and gallstones [2, 3, 4]. The husks are used for the treatment of pains and arthritis [5], ulcer [6], malaria and diabetes in Ibibio traditional medicine [7]. The husk extract has been reported to possess some pharmacological properties such as analgesic, anti-inflammatory [5], antioxidant [8], antidepressant [9], antimalarial and antiplasmodial [7], hepatoprotective [10], nephroprotective [11], antidiabetic and hypolipidaemic [12], and antiulcer [13] activities. The median lethal dose (LD_{50}) of the ethanol husk extract was determined to be 1874.83 mg/kg [9]. Arabinoxylan, which has immunological effects, has been isolated from the husk extract [14], while eight phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, ferulic acid, rutin, resveratrol, and kaempferol) have also been detected in ethanol husk extract of *Zea mays* [8]. Corn husk has also been reported to be rich in Anthocyannins [15]. In this study, we report the nephroprotective activity of the husk extract against gentimicin-induced kidney injury in rats to confirm its use in the treatment of kidney diseases in Ethnomedicine.

2. Materials and Methods
2.1 Collection of plant materials
Fresh husks of *Zea mays* were collected in August, 2018 from a farmland in Uyo, Uyo Local Government Area, Akwa Ibom State, Nigeria. The husks were identified and authenticated as *Zea mays* by a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPH, 614) was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.2 Extraction
The plant parts (husks) were washed, cut into smaller pieces and air-dried on laboratory table for 2 weeks. The dried husks were pulverized using electric grinder. The powdered husk (1.5 kg) was macerated in 50% ethanol for 72 hours. The liquid filtrate obtained was concentrated and evaporated to dryness in vacuo at 40 °C using rotary evaporator. The crude extract (yield 2.83%) was stored in a refrigerator at -4 °C until they were used for the experiments reported in this study.

2.3 Animals
Wistar male rats (150 - 165 g) used for these experiments were gotten from Animal house of Department of Pharmacology and Toxicology, University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH, 1996). Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

2.4 Effect of *Zea mays* husk extract on gentimicin-induced nephrotoxicity in rats
A total of 36 rats were used for this experiment and the design was as follows

Group 1: (Control group): Rats were orally administered 10 mL/kg body weight distilled water per oral for 8 days.

Group 2: (Organotoxic group): Rats were administered 10 mL/kg body weight distilled water for 8 days.

Group 3: (Standard group): Rats were administered 100 mg/kg body weight Silymarin per oral for 8 days.

Group 4: (Low dose test group): Rats were administered 187 mg/kg body weight of *Zea mays* husk extract orally for 8 days.

Group 5: (Middle dose test group): Rats were administered 347 mg/kg body weight *Zea mays* husk extract orally for 8 days.

Group 6: (High dose test group): the rats were administered 748 mg/kg body weight *Zea mays* husk extract orally for 8 days.

Gentimicin, 100 mg/kg body weight, was administered intraperitoneally daily to rats in groups 2-6 concomitantly with the above treatment for 8 days. Twenty four hours after the last administration, animals were weighed again and sacrificed under light diethyl ether vapour.

2.5 Collection of blood samples and organs
After 8 days of treatment (24 hours after the last treatment) the rats were weighed again and sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture and used immediately. Blood was collected into plain centrifuge tubes bottles, and were centrifuged immediately at 2500 rpm for 15 minutes to separate the serum at room temperature to avoid haemolysis and used for biochemical assays. The kidneys were surgically removed, weighed and fixed in 10% formaldehyde for histological process.

2.6 Kidney function test
The following biochemical parameters were determined as markers of kidney function using diagnostic kits at the Chemical Pathology Department of University of Uyo Teaching Hospital: Levels of electrolytes (Na, K, Cl, and HCO_3), creatinine and blood urea.

2.7 Histopathological analysis.
The kidneys of each animal that was used in the study were surgically harvested and fixed in buffered formalin. They were then processed and stained with haematotoxylin and eosin (H&E) for kidney study according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Morphological changes in the kidneys of the sacrificed animals were observed and recorded. Histologic pictures were taken as micrographs.

2.8 Statistical analysis
Data obtained from this work was analysed statistically using ANOVA (one –way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

3. Results
3.1 Nephroprotective effect of *Zea mays* husk extract against gentimicin-induced kidney injury.
Pretreatment of rats with the husk extract of *Z. mays* (187 – 748 mg/kg) prior to treatment with gentamicin was found to protect the animals from kidney injuries. Serum creatinine, serum urea and electrolytes (K^+, Na^+, Cl^- and HCO^-_3) levels were found to be significantly ($p< 0.05 – 0.001$) elevated in rats treated with only gentamicin when compared to normal control; whereas treatment with the husk extract significantly ($p< 0.05$-0.001) lowered their levels in the treated animals dose-dependently. Theer were significant ($p< 0.01$ - 0.001) and dose dependent reductions in the levels of serum creatinine and urea in the extract treated groups. 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 (Table 1).

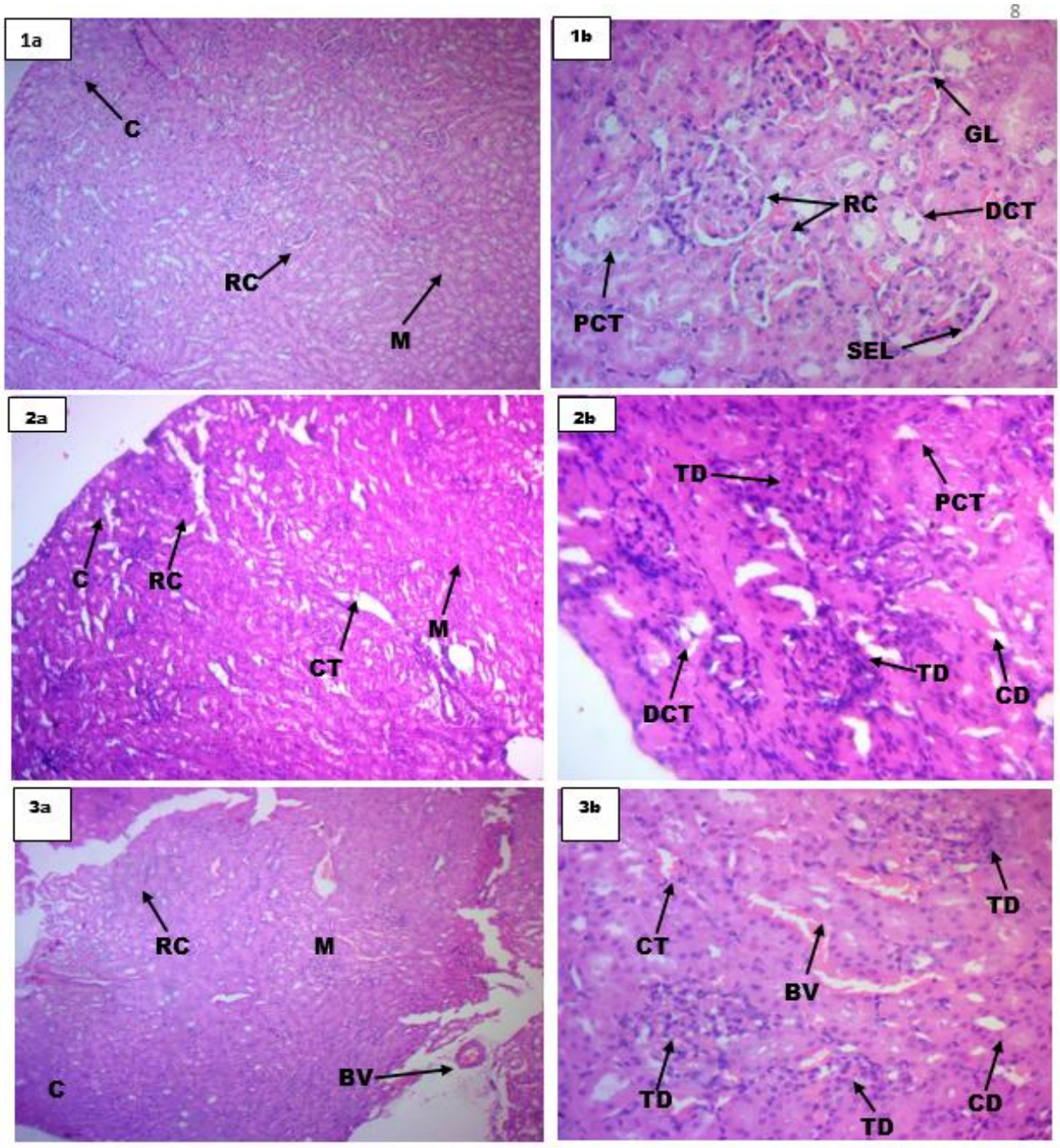
Table 1: Effect of husk extract of *Z. mays* on gentamicin-induced kidney injury in rats

Parameters/ Treatment	Creatinine (mg/dl)	Urea (mg/dl)	Bicarbonate (mmol/L)	Sodium ion (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Kidney weight (g)
Normal control	0.49±0.07	18.02±2.08	29.0±1.47	147.0±1.47	5.29±0.19	97.5±3.47	1.10±0.06
Gentimicin +Dist. Water	1.31±0.20^{c}	57.87±3.03^{c}	51.75±2.39^{c}	201.23±6.76^{c}	9.87±1.01^{c}	146.75±6.12^{c}	1.33±0.14^{a}
Silymarin (100 mg/kg)	0.82±0.04^{d}	49.15±3.94^{c}	54.75±2.28^{c}	192.5±2.87^{c}	7.30±0.37^{d}	132.25±2.62^{c}	1.70±0.11
Ext.187 mg/kg	0.68±0.05^{b}	44.42±1.96^{c,d}	45.75±1.25^{c}	181.5±1.55^{c,e}	6.53±0.23^{e}	124.0±3.39^{d}	1.14±0.06
Ext. 374 mg/kg	0.60±0.04^{f}	39.0±1.47^{c,f}	47.50±3.17^{c}	165.5±2.84^{a,f}	6.28±0.21^{f}	119.25±4.62^{a,e}	1.27±0.11
Ext. 748 mg/kg	0.56±0.02^{f}	29.37±0.44^{a,f}	35.55±1.78^{f}	161.0±1.78^{f}	5.66±0.20^{f}	107.0±6.79^{f}	1.20±0.18

Values are expressed as mean ± SEM. Significant at $^{a}p< 0.05$; $^{b}p<0.01$; $^{c}p< 0.001$ when compared to control. $^{d}p< 0.05$, $^{e}p< 0.01$, $^{f}p< 0.001$ when compared to gentimicin. n = 6.

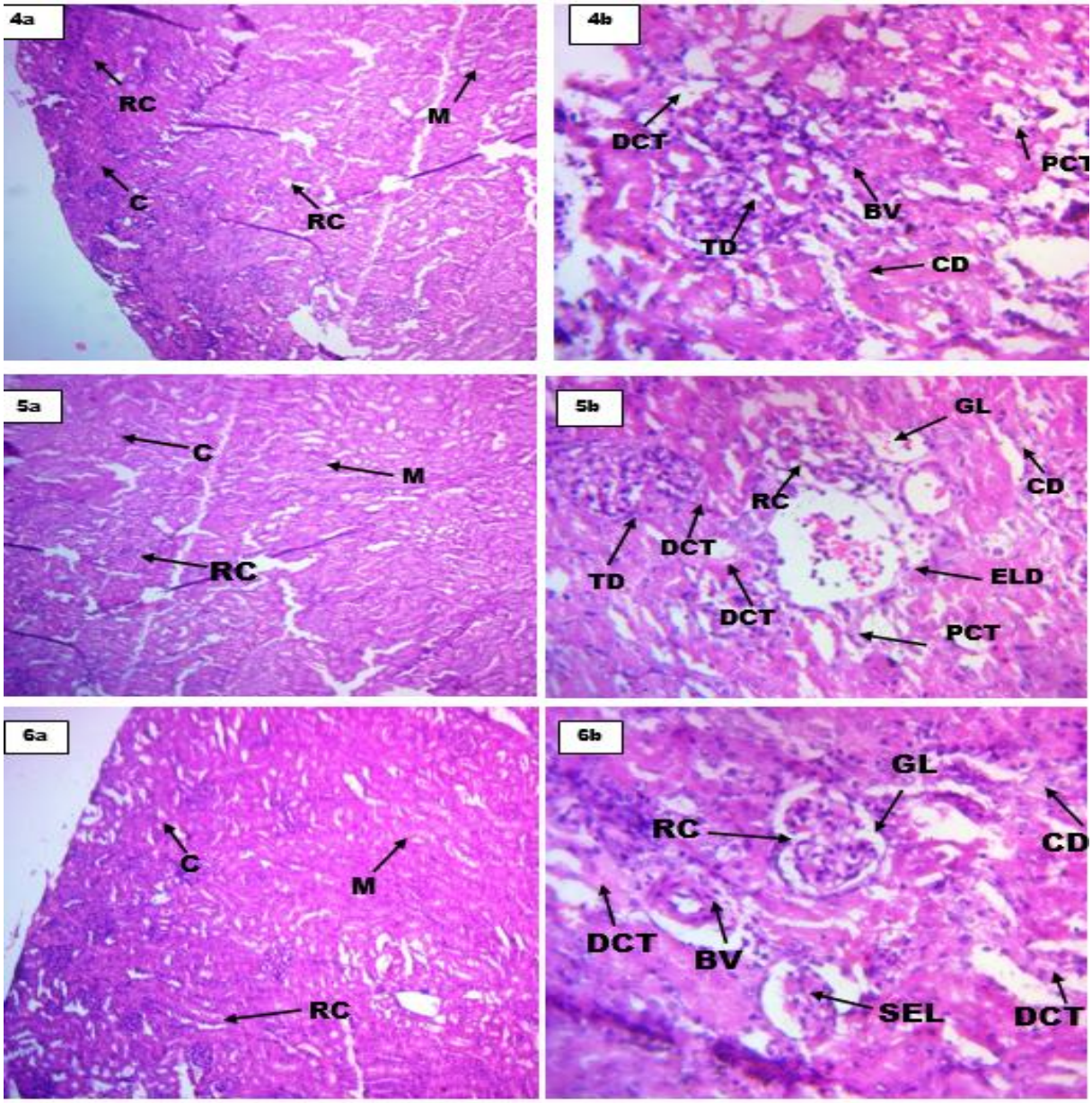
3.2 Histopathological studies of rat kidney in gentamicin-induced nephrotoxicity.

Histological study revealed that sections of kidney of rats administered distilled water (10 mL/kg) at magnification 1A (x100) and 1B(x400) revealed normal cortex and medulla regions with area of renal corpuscle containing glomerulus, lined with squamous epithelial lining, collecting tubules, proximal and distal collecting ducts, all within normal cellular architecture (Figures 1a and1b). Whereas, rats in the group treated with gentimicin only, showed various degrees of injuries such as oedematous glomerular with periarteriolar haemorhage, oedematous interstitium, thyroidization (colloidal casts), atrophic and degenerated glomerular, inflammatory cells infiltrate, tubular degeneration, congested and dilated capillaries, ruptured and degenerated glomerular (Figure 2a and 2b). Prior administration of cornhusk extract of *Z. mays* (187 - 748 mg/kg) and silymarin (100 mg/kg) were found to reduce such changes in kidney histology induced by gentamicin in rats (Figures 3a and 3b; 4a and 4b; 5a and 5b; 6a and 6b).



Keys: Renal corpuscle (RC), Convoluted tubules (CT), Squamous epithelial lining (SEL), Glomerulus (GL), Tubular necrosis (TN),Collecting ducts (CD), Cortex (C), Medulla (M)), Proximal Convoluted tubules (PCT), Distal Convoluted tubules (DCT), Squamous epithelial lining (SEL), Blood vessel (BV), Tubular degeneration (TD)

Fig 1-3: Showing: Histological sections of kidneys of rats treated with Normal saline 10 mL/kg (1a, 1b), Gentimicn 100 mg/kg (2a, 2b) and Silymarin 100 mg/kg and Gentimicn 100 mg/kg (3a,3b) at magnification A (x100) and B(x400) stained with H&E technique.



Keys: Renal corpuscle (RC), Proximal Convoluted tubules (PCT), Squamous epithelial lining (SEL), Glomerulus (GL), Epithelial lining degeneration (ELD), Convoluted tubules (CT), Squamous epithelial lining (SEL), Glomerulus (GL), Cortex (C), Medulla (M)), Proximal Convoluted tubules (PCT), Distal Convoluted tubules (DCT), Squamous epithelial lining (SEL), Blood vessel (BV), Tubular degeneration (TD), Collecting ducts (CD).

Fig 4-6: Showing Histological sections of Kidneys of rats treated with HE 187 mg/kg and Gentimicin 100 mg/kg (4a,4b), HE 374 mg/kg and Gentimicin 100 mg/kg (5a,5b) and HE 784 mg/kg and Gentimicin 100 mg/kg(6a,6b) at magnification A (x100) and B(x400) Stained with H&E technique.

4. Discussion

The present study was carried out to investigate the nephroprotective activity of *Zea mays* husk extract against gentimicn-induced kidney injury in rats. Effect of the husk extract on kidney function test and histology were used as parameters to assess this property. Gentamicin is nephrotoxic because a small but sizable proportion of the administered dose is retained in the epithelial cells of the proximal tubules after glomerular filtration [16]. This induces conspicuous 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 excretion [17]. Gentamicin also induces oxidative stress through induction of reactive oxygen species (ROS) such as free radicals, superoxide, hydroxyl radical anion and hydrogen peroxide [18]. When ROS are generated as a consequence to tissue injury induced by gentamicin, there is attack on different cell components 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 failure [18]. The effect of ROS in the body is usually suppressed by antioxidant enzyme systems. Gentimicin-induced kidney injury is presented by increase in serum levels of creatinine, urea, uric acid as well as severe proximal renal tubular necrosis followed by renal failure [19]. 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 husk extract of *Z. mays* produced prominent decreases in serum levels of creatinine, urea and

electrolytes induced by gentimicin. Also, the husk 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 gentimicin treatment when compared to normal control. The suppression of gentimicin-induced nephrotoxicity by the extract may have resulted from the antioxidant and free radical scavenging potentials of the extract.

Histopathologically, in H&E stain observation, there was significant nephroprotective potential by the cornhusk extract with slight features of damage to area of epithelial lining degeneration, glomerulus, and tubules when compared with the gentamicin group that showed nucleus. This result agrees with other parameters that cornhusk extract had a dose dependent nephroprotective effect against gentamicin induced toxicity. The plant may therefore act against gentamicin-induced toxicity by suppressing the activities of ROS through its antioxidant contents, thereby preventing many degenerative processes and restoring homeostasis of biological electrolytes.

The protection is due to the free radical scavenging potentials of the phytoconstituents of the extract and fractions such as anthocyannins, stigmasterol, sitosterol, *p*-hydroxycinnamic acid and octadecanoic acid [20, 21, 22, 23, 24, 25, 26] as well as the antioxidant activity of other phenolic compounds present in the extract8. This finding corroborates that of [11] who reported nephroprotective and antioxidative stress activities of the cornhusk in diabetic rats which were attributed to the presence of anthocyanins and their antioxidant property.

The data generated from these experiments has provided the scientific basis for the wide use of this plant as traditional agent for treating kidney diseases. The results of this study offer a platform of using corn husk as an antidote and in treatment of kidney diseases.

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6. Conflict of Interest

We declare that we have no conflict of interest.

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