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Nephroprotective and Curative Assessment of an Aqueous Seed Extract of *Parkia clappertoniana* Keay in Gentamicin-induced Renal Damage in Sprague-dawley Rats

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Authors' contributions

Authors AB and GAK designed the study, managed the analysis of the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors JA, EOA and LFO collected data, managed the literature searches, and performed laboratory experiments. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To assess the nephroprotective and curative effects of an aqueous seed extract of *Parkia clappertoniana* on gentamicin-induced renal damage (GIRD) in Sprague-Dawley rats.
Study Design: Experimental
Place and Duration of Study: Department of Medical Laboratory, University of Cape Coast, Ghana between September, 2012 and May 2013.
Methodology: In assessing nephro protective effect, rats were pretreated (10 days) with *P. clappertoniana* aqueous seed extract (1-2 g kg^{-1}; p.o) prior to induction of renal damage by treatment with gentamicin (0.08g kg^{-1}; p.o, for 8 days. Serum biochemical markers (Creatinine, Urea, Na^+ and K^+) and urine parameters (leukocyte, protein, specific gravity and pH) of renal damage were determined and compared with baseline values. In a curative study, GIRD in rats was treated with Normal Saline (2 ml kg^{-1}; p.o), Losartan

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(0.05 g kg^{-1}; p.o), or extract (1-2 g kg^{-1}; p.o) for 14 days and serum and urine parameters determined for all treatments. Histopathology and changes in kidney weights for normal and treated rats in both studies were assessed. The extract was screened for DPPH radical scavenging activity.
Results: The extract significantly ($P \leq .001$) reduced elevated serum creatinine and urea secondary to GIRD ($P \leq .05$) and significantly ($P \leq .05$) reduced elevated serum Na^+ but had no effect on K^+. Elevated urine proteins and leucocytes secondary to GIRD was significantly ($P \leq .05$) reduced; but had no significant effect on urine pH and specific gravity. Elevated kidney weights associated with GIRD was significantly ($P \leq .01$) reduced. Histopathological assessment revealed healing effect by extract to GIRD. Effects of the extract were similar to Losartan. Pretreatment with extract however had no significant effect on GIRD as serum and urine parameters, as well as kidney weights were significantly ($P \leq .01$) elevated on induction of renal damage.
Conclusion: The aqueous seed extract of *Parkia clappertoniana* has curative but no nephroprotective effect on gentamicin-induced renal damage in Sprague-Dawley rats.

Keywords: Plasma creatinine; Plasma urea; Azotemia; Proteinuria; Hypersthenuria; reactive oxygen species; Saponins; Polyuronides.

1. INTRODUCTION

Chronic renal disease (CRD) is the progressive loss of kidney function over time. Worldwide prevalence of CRD has increased dramatically over the past decades and epidemiological reports support this fact (New York National Kidney Foundation, 2007). An estimated 26 million United States adults are reported suffering from CRD i.e. 1 out of every 9 Americans has CRD [1]. A recent report had revealed an epidemic in Central America, where more than 16,000 people (mostly sugarcane workers) died from an incurable CRD [2]. Studies have shown that, the prevalence of CRD has reached epidemic proportions with 10-13% of the populations of China, Taiwan, Canada , India and Iran still showing signs and symptoms of CRD [3]. In Ghana, CRD is reported to be on the increase, with the Renal Unit of the Medical Department of the Korle-Bu Teaching Hospital, Accra, Ghana, recording 3,281 cases in 2010; a 38 % increase over the cases recorded in 2009 [4].

Schieppati and Giuseppe in 2005 [5] had reported the major complications of CRD to be cardiovascular and cerebrovascular diseases and that victims are more likely to die of cardiovascular disease than to develop a terminal renal failure. Other complications that arise from CRD may include decreased glomerular filtration rate leading to anemia, malnutrition, bone and mineral disease [6].

Presently, management and treatment for CRD is quite unavailable particularly in low income countries and where treatment is available it is costly especially for the poor. A typical case is that of Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana, where a report indicates that 96% of patients with CRD attending KATH could not afford medications, leading to treatment non-compliance [7]. Some of the available treatments include hemodialysis, pharmacotherapy (Use of drugs e.g. folate-conjugated rapamycin, Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers) and dietary interventions (low fluids, potassium, phosphates, sodium and proteins) [8].

Though these treatments have been proven to slow the rate of progression of CRD, they do not completely treat it [8], and even more disturbing is that they are quiet costly as previously

mentioned necessitating the need to vigorously search for cheaper but effective sources of alternative and adjunct drugs which have renoprotective (i.e. protecting the kidney against harmful effects, such as of a drug or other chemicals [xenobiotics]) and/or curative effect(s) which is affordable to the lower income majority of the Ghanaian population. It is in pursuance of such alternative agents that we investigated an aqueous seed extract of *Parkia clappertoniana* (Family: Fabaceace–mimosoideae), known commonly in English as Clapper`s parkia.

Parkia clappertoniana is commonly known as African locust bean tree. It is a perennial deciduous tree distributed in varied agro ecological zones ranging from tropical forest to arid vegetation zones. It is common in many African countries including Ghana. In Ghana the seeds are fermented mostly by Northern tribes to form a local condiment called *dawadawa*, which is used in the preparation of various foods as a seasoning agent or flavour. *Parkia clappertoniana* has demonstrated many pharmacological activities traditionally including hepatoprotective effect [9]; anti-diabetic and anti-hyperlipidaemic effect [10]; anti-bacterial properties [11]; effective against leprosy, sore eyes, bronchitis, ulcers and skin infections [12] and effective against hypertension [13], an underlying cause of CRD. It has nutritive properties [14,15] and contains cardiac glycosides, steroids, tannins and alkaloids [11,16].

The aim of this study therefore is to assess the effect of an aqueous seed extract of *Parkia clappertoniana* on gentamicin-induced renal damage in Sprague-Dawley rats using curative and prophylactic treatment regimes.

2. MATERIAL AND METHODS

2.1 Collection and Authentication of *Parkia clappertoniana* seeds

The seeds of *Parkiaclappertoniana* were collected from Wa, the capital of the Upper West Region of Ghana. It was identified and authenticated by a plant taxonomist at the herbarium unit, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana where a voucher specimen (UCC/SBS/P133) was deposited.

2.2 Preparation of *Parkia clappertoniana* Aqueous Seed Extract

A 800 g quantity of *P. clappertoniana* seeds were boiled to aid removal of the husk leaving the beans. The beans, weighing 645 g, were wrapped in polyethene bags for 4 days to ferment. The fermented beans were then sun dried, pulverized and steam-boiled in 1.75 L of distilled water for 30 min. On cooling, the mixture was filtered with What man's No. 1 filter paper (Whatman / Schleicher &Schuell, GmbH, Austria). The filtrate, in a round-bottomed flask, was frozen in a pre-freezer (S-IF, Rikakikai Co. Ltd Tokyo, Japan) and mounted on a Heto Power Dry LL 300 freeze-dryer (Thermo Electron Corporation, USA) for 6 days until the extract was fully dried (Percentage yield: 24.5%). The extract was then transferred into a plastic container and labelled APC for use in this study.

2.3 Phytochemical Screening of APC

Preliminary phytochemical screening was conducted on APC using standard phytoanalytical methods as described by [17], and [18].

2.4 Drugs and Chemicals Used

Gentamicin (Tivagenta, Greenfield Pharmaceutical, Jiang Su, China) was used to induce renal damage in experimental animals. Buffered formalin [4% formaldehyde in buffered isotonic saline] (Reagents, USA) was used to preserve the isolated kidneys, Losartan, (an angiotensin converting enzyme –inhibitor) was used as a reference drug.

2.4 Experimental Animals

Eight weeks old healthy Sprague-Dawley ratsof either sex weighing (240 - 270g) were used for the study. Theywere purchased from the Center for Scientific Research into Plant Medicine (CSRPM), Mampong-Akuapem, Ghana. The rats were fed on pelleted feed (AGRICARE Ltd, Kumasi, Ghana) and allowed free access to clean water. Animals were kept under ambient conditions of light/dark, humidity and room temperature throughout the study. The "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

2.5 Experimental Conduct and Design

2.5.1 Gentamicin-induction renal damage (GIRD)

Renal damage was induced using the method described by Sakthi et al.,[19], and Eslami et al.,[20] with some modification. Rats were injected gentamicin (0.08 gkg^{-1}, *i.m*) daily for 8 days. On day 9, urine and blood samples (taken from the jugular vein) were taken for analyses. The kidneys were isolated from sacrificed animals, weighed and preserved in 10 % buffered formalin for histopathological studies. Confirmation of renal damaged was done by an earlier trial study where 5 rats were treated with gentamicin for 8 consecutive days, on day 9, urine and blood samples were collected for estimation of serum and urine biochemistries and also kidney pathological examinations. All the rats showed elevated serum biochemistry (Urea, creatinine, Na^+ and K^+), elevated levels of urine proteins and leucocytes, elevated kidney weights, and Histopathological changes in kidney microstructure.

2.5.2 Nephroprotective assessment

Twenty one Sprague-Dawley rats were grouped into 3 (A-C) with seven animals per group. Baseline urine (leukocyte, protein, specific gravity and pH) and serum (creatinine, urea, Na^+ and K^+) parameters of kidney damage, and kidney weights were determined from six rats (two selected at random from each group). Group A was then treated with 2 ml/kg Normal Saline while Groups B and C were treated with 1 and 2 g kg^{-1} APC, *per os*, respectively for 10 days. Rats were then treated with gentamicin to induced renal damage. On the ninth day semi-quantitative urinalysis and serum biochemical analysis, kidney weights and histopathological assessments were again determined.

2.5.3 Curative assessment

In a separate experiment twenty eight Sprague-Dawley rats were randomly grouped into 4 (D-G) with seven animals per group. Baseline measurements were done as previously described by randomly selecting two rats from each group leaving each group with five rats each. Renal damage was induced after which groups were treated with either 2ml kg-1

Normal saline (D), 0.05 g kg^{-1} Losartan *per os* (E),or 1 and 2 g kg^{-1} APC *per os* (F and G respectively) for 14 days. Semi-quantitative urinalysis and serum biochemical analysis, kidney weights and histopathological assessments were determined.

2.5.4 Semi-quantitative urinalysis

Urine samples were collected by using the method of Khosho et al., (1985) [21] with some modification. Measurement of urinary pH, protein, leucocytes and specific gravity were done by using urine reagent strips (Accubiotech Co., Ltd, China). By using dipstick technique, and one reagent strip for one urine sample, each reagent strip was dipped into urine sample collected in small test tubes allowed to stay for 3 minutes then colour changes developed on the urine test strip was compared to standard colour charts provided by the test kit manufacturer to determine the results either qualitatively or semi-quantitatively.

2.5.5 Serum biochemical analysis

Blood samples were collected into MediPlus K3 EDTA tubes (Sunphoria Co. Ltd., Taiwan), allowed to coagulate and centrifuged (Denley BS 400, England) at 3000 rpm for 10 minutes to separate the formed elements from the serum. The serum obtained was stored in cap tight microtubes, labeled accordingly. Serum urea and creatinine were measured by using ELITech Clinical Systems protocol (Sees, France) whiles sodium and potassium were measured by using Beacon Diagnostics protocol (PVT Ltd. India) in a clinical chemistry analyzer (Vital Scientific N. V, Netherlands).

2.5.6 Histopathological assessment

The harvested kidneys were preserved in 10% buffered formalin and sent to the Pathology Department of the Noguchi Memorial Institute for Medical Research (NMIMR), Accra, Ghana for histopathological assessment. The tissues were processed by using standard procedures involving dehydration with a progressively increasing concentration of ethanol (50%-70% to 100% ethanol). The tissues were cleared with chloroform and impregnated with paraffin wax. Sections were made, stained with hematoxylin and eosin and mounted on slides for microscopic examinations and histopathological interpretation by a Pathologist

2.5.7 Antioxidant activity of APC

Scavenging activity of APC against 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was evaluated as described by Stankovic et al., 2010. A stock solution of APC prepared in methanol to a concentration of 100 µg ml-1 was diluted. Dilutions were made to obtain concentrations of 80 µg ml-1, 40 µg ml-1, and 20 µg ml-1. Each milliliter of the diluted solutions was mixed with 1ml of DPPH methanolic solution (40 µg ml-1). After 30 minutes in darkness at room temperature (23ºC), the absorbance of the test and blank samples were recorded at 517 nm using a microplate reader (Rayto RT-2100C, China). The control sample contained all the reagents except the extract. All experiments were performed thrice and the results were averaged. Percent inhibition was calculated using the following expression:

$$\% \text{ inhibition} = \frac{\left(A_{blank} - A_{sample}\right) \times 100}{A_{blank}}$$

Where and stand for absorption of the blank sample and absorption of tested extract solution respectively. The IC50 values which denote the concentration of test drugs required

to scavenge 50% of DPPH free radicals were estimated. Ascorbic acid was used as the standard.

2.6 Data Analysis

Data presented and plotted were mean ± SEM. The significance in the difference between measured parameters was established using the unpaired t-test (two-tailed) or One-Way Analysis of Variance followed by Dunnett's Multiple Comparisons Test *post hoc*. $P \leq .05$ was considered statistically significant.

3. RESULTS

3.1 Phytochemical Analysis

Phytochemical analysis of APC showed the presence of Saponins and Polyuronides.

3.2 Urinalysis

GIRD resulted in significant elevation ($P \leq .0001$) in urine leucocytes and proteins with a significant reduction in urine pH ($P \leq .0001$). APC (1-2 gkg^{-1} *p.o*) pre-treatment have no significant effect ($P > .05$) on GIRD as these parameters were still elevated ($P \leq .0001$) (Table 1). The curative study however showed that APC (1-2 gkg^{-1} *p.o*) and Losartan (0.05 gkg^{-1}) decreased significantly ($P \leq .0001$) elevated urine leucocyte and protein secondary to GIRD and normalized urine pH. Comparison of treatments and baseline values show no significant differences ($P > .05$). Treatment with normal saline had no significant effect as post-GIRD parameters remained elevated (Table 2).

3.3 Serum Biochemical Analysis

APC (1-2 gkg^{-1} *p.o*) pre-treatment in mice had no significant effect ($P > .05$) on GIRD as elevated serum creatinine, urea, and potassium secondary to GIRD were still high ($P \leq .0001$), but significantly reduced ($P \leq 0.001$) elevated sodium. Results were comparable to Normal saline (vehicle) treatment (Table 3). APC (1-2 gkg^{-1}; *p.o*) and Losartan treatment, in the curative assessment, significantly ($P \leq .001$) decreased elevated serum creatinine, urea, potassium, and sodium secondary to GIRD. Normal saline treatment did not have any effect on GIRD as serum creatinine, urea, potassium, and sodium were still high ($P \leq .001$) after treatment (Table 4).

3.4 Organ Weight-to-Body Weight Ratio

GIRD produced a marked increase in the wet weights of kidneys leading to elevated ($P \leq .001$) kidney weight-to-body ratios. Curative APC (1-2 gkg^{-1} *p.o*) and Losartan (0.05 gkg^{-1}) treatments however significantly reduced the wet kidney weights leading to significant decrease ($P \leq .001$) in kidney weight-to-body ratios. Pretreatment of rats with APC (1-2 gkg^{-1} *p.o*) did not prevent increase ($P > .05$) in wet kidney weights associated with GIRD. Normal saline treatment had no significant effect on elevated kidney weight associated with GIRD (Table 5).

3.5 Histopathological Assessment

Histopathological examination of kidneys in GIRD in Sprague-Dawley rats treated with 0.05 gkg^{-1} Losartan (Fig. 1c) and 1-2 gkg^{-1} APC (Figure 1 d and e) in the curative assessment showed progressive repair and correction of renal damage, reported as loss of cuboidal epithelial cells, vascular congestion in glomeruli with patchy focal chronic inflammation, and general architectural distortions of renal micro-structure, when compared to GIRD treatment with normal saline (Fig.1b). Pre-treatment of normal rats with 2 gkg^{-1} APC before GIRD could not prevent renal damage (figure 1f).

3.6 Antioxidant activity of APC

The DPPH radical antioxidant test was carried out on both APC and Ascorbic acid (Standard) indicated that both drugs caused a concentration dependent percentage increase in antioxidant activity. However, ascorbic acid had a more significant antioxidant activity as indicated by a comparatively low IC_{50} value (Fig. 2).

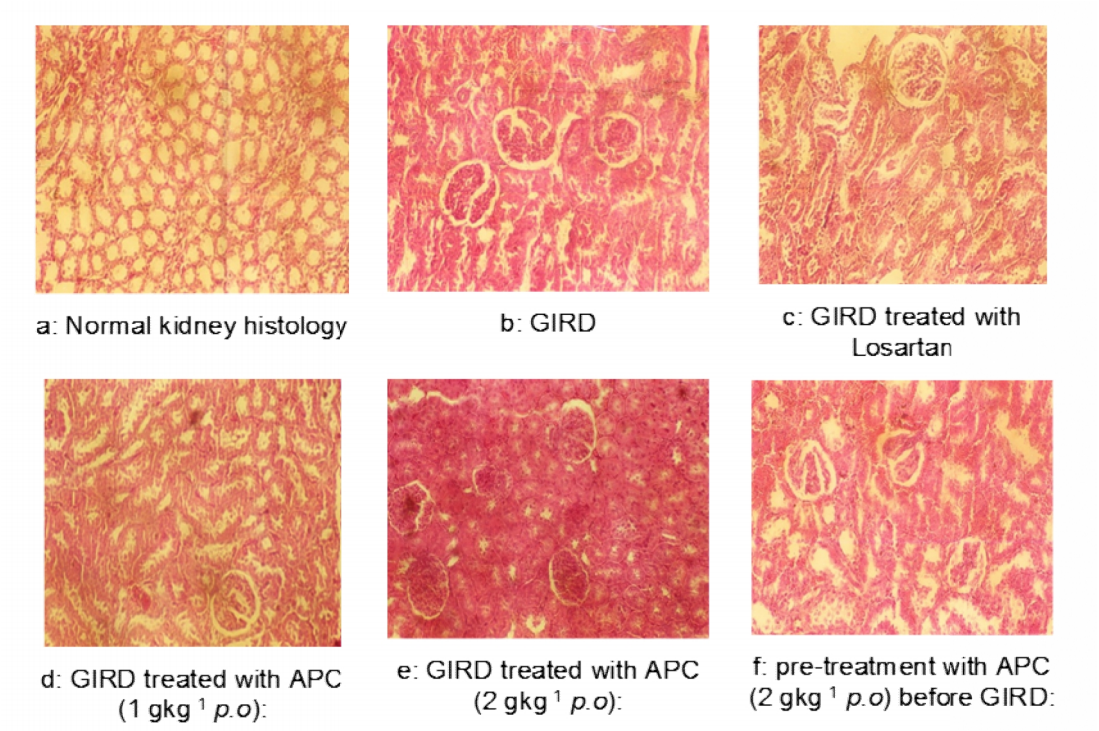

Fig. 1. Photomicrographs of the transverse section of the kidney of normal, entamicin-induced renal damage (GIRD), and GIRD with APC and Losartan treatment in Sprague-Dawley rats Hematoxylin and eosin (H & E) stain. X20

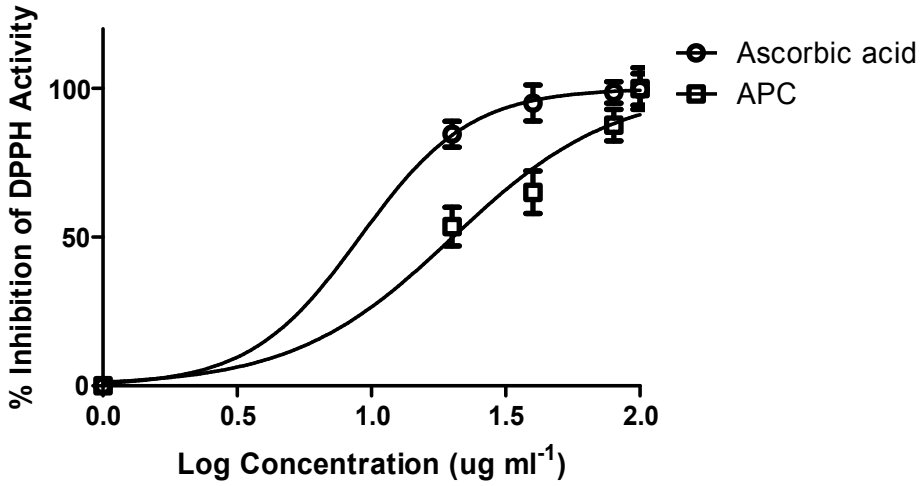

Fig. 2. Antioxidant activities of APC and ascorbic acid (Standard) expressed as % inhibition of DPPH free radical oxidant activity. Value plotted are mean ± SEM (n = 3). IC_{50} for Ascorbic acid and APC are 9.07 and 20.14 ug ml^{-1} respectively.

4. DISCUSSION

In the present study APC administered curatively decreased significantly azotemia (i.e. elevated serum creatinine and urea), hypersthenuria (elevated SG and solute concentration), and proteinuria, as well as elevated leukocytes secondary to GIRD in a manner similar to Losartan. These effects were not seen in rats pretreated with APC prior to GIRD. The functional status of a kidney is determined indirectly by serum and urine estimates of creatinine, urea and some electrolytes (Na^+, K^+, Cl^+). The ability of APC to dose-dependently reduce elevated levels of these renal biomarkers indicates that APC has curative effect.

The underlying mechanism of action of gentamicin has been linked to the formation of reactive oxygen species (ROS), which has been shown to increase with gentamicin administration [22]. The production of ROS stimulate the activation of pro-inflammatory mediators, including NF- *κ*B, leukocyte adhesion molecules, and mitogen-activated protein kinases (MAPKs) [23], which contribute to progressive kidney damage. Recent studies have shown that redox-sensitive transcription factors, MAPK and NF-kB, are involved in nephrotoxicity caused by gentamicin [24,25]. NF-*κ*B is a highly conserved family of transcription factors with a critical role in mediating inflammation, apoptosis, and progression of chronic disease [26]. Activation of NF-*κ*B, in response to oxidative stress might play a role in gentamicin-induced nephrotoxicity by inducing synthesis of inflammatory substances (cytokines, growth factors, and adhesion molecules) that provoke kidney damage [27]. In the present study, gentamicin administered to rats produced a typical pattern of renal damage which was manifested by marked increase in serum creatinine and urea levels. This had been confirmed in other studies which used gentamicin [20,28,29]. Thus, agents capable of reversing gentamicin-induced nephrotoxicity are believed to block NF-*κ*B interrupting the cascade of biochemical events that lead to renal damage.

Table 1. Urinalysis results for control (Baseline), Normal Saline (NS) and APC pretreatment prior to GIRD in assessing nephroprotective effect of APC in Sprague-Dawley rats

	TREATMENT GROUPS				
			Pretreatments before GIRD		
	Baseline	GIRD	2 ml kg^{-1} NS	1 gkg^{-1} APC	2 gkg^{-1} APC
Leucocytes (μL^{-1})	5.2 ± 0.88	405.0 ± 72.6$^{\phi\phi\phi}$	350 ± 91.8†††	415.0± 86.7 †††	394.0 ± 63.8 †††
Proteins (gL^{-1})	4.4 ± 0.95	30.0 ± 6.01$^{\phi\phi\phi}$	22.8 ± 4.42†††	26.14 ± 6.02 †††	24.1 ± 4.81 †††
SG	1.026 ± 0.0023	1.089 ± 0.0062$^{\phi\phi\phi}$	1.082 ± 0.0043 †††	1.092 ± 0.007 †††	1.088 ± 0.0056†††
pH	6.4 ± 0.28	5.2 ± 0.12$^{\phi\phi\phi}$	5.6 ± 0.32†††	5.4 ± 0.20†††	5.2 ± 0.25 †††

Values are means ± SEM (n = 5; Baseline: n=6). Difference between Baseline and GIRD: $^{\phi\phi\phi}$ P ≤ 0.0001 (unpaired t-test (two-tailed). Differences between GIRD and pre-treatment groups were not significant: P > 0.05; Differences between Baseline and pre-treatment groups: ††† P≤ 0.0001 (One-Way ANOVA followed by multiple Dunnett's post hoc test). Baseline represents normal rats (no induction of renal damage, no treatments). SG = Specific gravity; GIRD = Gentamicin-Induced renal damage.

Table 2. Urinalysis results of the effect of Normal saline (NS), Losartan (LOS), and APC treatment after GIRD in a curative assessmentin Sprague-Dawley rats

	TREATMENT GROUPS					
			Treatments after GIRD			
Parameters	Baseline	GIRD	2 ml kg^{-1} NS	0.05gkg^{-1} LOS	1 gkg^{-1} APC	2 gkg^{-1} APC
Leucocytes(μL^{-1})	4.4 ± 0.87	425 ± 75.0$^{\phi\phi\phi}$	375 ± 91.8†††	7.1± 0.76 ***	6.0 ± 0.95 ***	4.8 ± 0.96 ***
Proteins (gL^{-1})	3.6 ± 0.92	26.6 ± 3.4$^{\phi\phi\phi}$	28.9 ± 4.5†††	6.06 ± 0.94***	5.4 ± 0.66 ***	7.2 ± 0.83 ***
SG	1.028 ± 0.008	1.092 ± 0.0064$^{\phi\phi\phi}$	1.083 ± 0.004†††	1.025 ± 0.006 ***	1.031 ± 0.0061 ***	1.023 ± 0.003 ***
pH	6.9 ± 0.33	5.6 ± 0.19 $^{\phi\phi\phi}$	6.1 ± 0.12†	7.0 ± 0.28**	6.8 ± 0.30**	6.9 ± 0.26 **

*Values are means ± SEM, (n=5, Baseline: n=10). Differences between Baseline and GIRD: $^{\phi\phi\phi}$ P≤ 0.001(unpaired t-test (two-tailed). Significant differences between GIRD and treatments: ** P ≤ 0.01, *** P ≤ 0.001. Differences between baseline and treatments: † P ≤ 0.05; ††† P ≤ 0.001 (One-Way ANOVA followed by multiple Dunnett's post hoc test). Baseline represents normal rats (no induction of renal damage, no treatments). SG = Specific gravity; GIRD = Gentamicin-Induced renal damage.*

Table 3. Serum biochemistry results for control (Baseline), Normal Saline (NS) and APC pretreatment prior to GIRD in a nephroprotective assessment in Sprague-Dawley rats

	TREATMENT GROUPS				
			Pretreatment before GIRD		
Parameters	Baseline	GIRD	2 ml kg^{-1} NS	1 gkg^{-1} APC	2 gkg^{-1} APC
Cr (µmol/l)	26.35 ± 8.81	81.86 ± 9.45 $^{\phi\phi\phi}$	72.12 ± 7.01†††	69.26 ± 8.67†††	74.57 ± 9.30 †††
Ur (mmol/l)	6.54 ± 0.57	19.22 ± 2.75$^{\phi\phi\phi}$	19.12 ± 1.52†††	16.77 ± 2.42 †††	16.8 ± 1.64†††
Na^+ (mmol/l)	119.31 ± 29.40	265.50 ± 12.68 $^{\phi\phi}$	243.02 ± 30.12	91.24 ± 17.08 ***	79.70± 29.88 ***
K^+ (mmol/l)	6.74 ± 0.54	14.95 ± 1.68$^{\phi\phi\phi}$	15.43 ± 2.82	16.56 ± 1.53 †††	17.50 ±2.48 †††

*Values are mean ± SEM (n = 5; Baseline: n=6). Differences between baseline and GIRD: $^{\phi\phi}$ P ≤ 0.001, $^{\phi\phi\phi}$ P ≤ 0.0001,(unpaired t-test (two-tailed). Differences between GIRD and pre-treatment groups: *** P ≤ 0.001. Differences between Baseline and pre-treatment groups: ††† P≤ 0.001 (One-Way ANOVA followed by multiple Dunnett's post hoc test). (One-Way ANOVA followed by multiple Dunnett's post hoc test). Cr = Creatinine; Ur = Urea. GIRD = Gentamicin-induced renal damage*

Table 4. Serum Biochemistry results of the effect of Normal saline (NS), Losartan (LOS), and APC treatment after GIRDin a curative assessment in Sprague-Dawley rats

	TREATMENT GROUPS					
			Treatments after GIRD			
Parameters	Baseline	GIRD	2 ml kg^{-1} NS	0.05gkg^{-1} LOS	1 gkg^{-1} APC	2 gkg^{-1} APC
Cr(µmol/l)	28.35 ± 2.81	85.57 ± 10.30$^{\phi\phi\phi}$	80.66 ± 2.21 †††	26.56 ± 1.32 ***	32.76 ± 2.39***	27.22 ± 3.05 ***
Ur(mmol/l)	6.94 ± 0.87	18.77 ± 1.42$^{\phi\phi\phi}$	16.02 ± 0.88†††	9.82 ± 0.65***,	9.30 ± 0.92 ***	7.37 ± 0.75***
Na^+(mmol/l)	106.85 ± 25.69	280.23 ± 19.04$^{\phi\phi}$	260.42 ± 18.09 †††	154.73 ± 12.87***	135.00± 20.02***	130.73±7.87***
K^+(mmol/l)	7.14 ± 0.68	15.80 ± 1.02$^{\phi\phi\phi}$	14.65 ± 0.87 †††	9.65 ± 0.57 **	8.16 ± 0.80***	7.65 ± 0.63 ***

*Values are mean ± SEM (n = 5; Baseline: n=10). Differences between baseline and GIRD:$^{\phi\phi}$ P ≤ 0.001, $^{\phi\phi\phi}$ P ≤ 0.0001(unpaired t-test (two-tailed). Differences between GIRD and treatment groups: *** P ≤ 0.001. Difference between Baseline and treatment groups: ††† P ≤ 0.001 (One-Way ANOVA followed by multiple Dunnett's post hoc test). Cr = Creatinine; Ur = Urea. GIRD = Gentamicin-Induced Renal Damage.*

Table 5. Effect of Gentamicin, APC, and Losartan treatments on wet kidney weight-to-body weight ratio in a nephroprotective, and curative assessment in Sprague-Dawley rats

Treatments	Pretreatment	GIRD	Post-treatment
Baseline	0.004 ± 0.0003	0.009± 0.0005$^{\phi\phi\phi}$	
1 gkg^{-1}APC^{t}	0.004 ± 0.0003	0.008 ± 0.0004***	
2 gkg^{-1}APC^{t}	0.003 ± 0.0002	0.008 ± 0.0004***	
2 ml kg^{-1} NS		0.008 ± 0.0004	0.008 ± 0.0003
0.05gkg^{-1}LOS		0.009 ± 0.0005	0.005 ± 0.0002†††
1 gkg^{-1} APC		0.008 ± 0.0003	0.005 ± 0.0003†††
2 gkg^{-1} APC		0.009 ± 0.0004	0.005 ± 0.0002†††

*Values are means ± SEM (n=5, Baseline: n=16). ^{t}APC pre-treatment. Difference between Baseline and gentamicin-induced renal damage (GIRD); $^{\phi\phi\phi}$ P ≤ 0.001. Differences pretreatments and GIRD: *** P ≤ 0.001. Differences between GIRD and post-treatment: ††† P ≤ 0.001 (Unpaired t-test (two-tailed).*

Gentamicin-induced renal damage is normally characterized by Tubular necrosis [30]. Additionally, gentamicin has been shown to induce proximal tubular injury, with the renal damage ranging from alterations in tubular reabs orption, loss of renal microstructure to necrosis of proximal tubule cells [31,32]. Mechanistically many reports have indicated that gentamicin stimulates mesangial cell contraction and proliferation which leads to reduced renal function and the elevation of electrolyte concentration and serum creatinine and urea[30, 33].Increase in serum creatinine and lowered renal clearance has been linked to chronic renal failure [33,34]. [35] had even suggested that lysosomal membrane rupture and release of acid hydrolases contribute to apoptosis and necrosis of proximal tubular cells, and this has been demonstrated pathologically.

Normal kidney sections showed cuboidal epithelial cell lining of the tubules. GIRD resulted in tubular epithelial damage with intense granular degeneration involving the renal cortex. This brought about a structural distortion in the kidney architecture. The presence of the tubular necrosis, epithelial loss and degeneration of glomeruli had also been reported [28,29].

A lowering of elevated kidney weight associated with GIRD confirmed the curative effect of APC treatment. Gentamicin-induced loss of protein particularly albumin leading to hypoalbuminemia in renal blood vessels reflexively provoked the compensatory hemodynamic system of the kidney to conserve water by reabsorbing Na^{+}. Also, because of cellular injury many inflammatory cells and mediators were drawn in leading to fluid accumulation and further cellular congestion. Because the mesangial cell network, which maintains the vasoelasticity of the kidney is disrupted already by gentamicin coupled with necrosis and apoptosis fluid retains in the kidneys leading to kidney hypertrophy and increased kidney weight. The ability of APC to reduce hypernatremia secondary to gentamicin-induced renal damage underscores the potential of APC to reverse kidney damage. APC had no effect on K^{+} perhaps the reductive effect of APC was counteracted by reduced excretion of K^{+} aggravated by leakage of intracellular potassium into the blood stream as a result of lesions in renal tubular epithelium secondary to GIRD treatment [36,37].

The above observations were confirmed by histopathological examinations of the isolated kidneys and also the organ-body weight ratios. For instance GIRD treated rats showed morpho pathological alterations, including: a diminished Bowman's capsule, apoptosis and cellular necrosis, mesangial proliferation, tubular obstruction, and basal membrane

interruption; however following APC treatment these histopathological alterations were ameliorated. Again, GIRD treated rats showed high organ-body weight ratios but these were significantly reduced following APC and losartan treatments.

Losartan, a selective, competitive angiotensin II receptor type 1 receptor antagonist and APC significantly reversed GIRD. LOS has antioxidant activity. It significantly attenuated Gentamicin-induced increase in malondialdehyde and decrease in reduced glutathione, and catalase and superoxide dismutase activities in renal cortical homogenates [38]. Malondialdehyde is an oxidative stress biomarker; particularly lipid per oxidation [39]. Glutathione is an important intracellular antioxidant that protects against a variety of different antioxidant species [40]. Catalase and superoxide dismutase are enzyme present in most of the aerobic cells, which protects them from oxidative stress [41,42].

The curative effect of APC could possibly have been due to the collective antioxidant effect of all the secondary plant metabolites i.e. phytochemicals present in the extract which were saponins and polyuronides. Triterpenoids and steroidal glycosides, collectively referred to as saponins are bioactive compounds present naturally in many plants. Saponins are a major family of secondary plant metabolites containing a sugar moiety glycosidically linked to a hydrophobic aglycone (sapogenin). Polyuronides are natural antioxidants which function as terminators of free radical chains or as chelators of redox-active metal ions capable of inhibiting lipid per oxidation [36,37].

Many reports [43-46] had demonstrated the antioxidant and anti-inflammatory pharmacological activities of saponins in both *in vitro* and *in vivo* experiments. Even [47] have reported the chemo protective effects of saponins. Overproduction of ROS has been considered to mediate in part many toxic reactions in living systems, and has specifically been shown in gentamicin-induced nephrotoxicity [22,23]. Therefore, compounds that can scavenge ROS have great potential in ameliorating these toxic reactions. Saponins and polyuronides in APC possibly acted synergistically or additively in scavenging ROS, thus played an important role in ameliorating gentamicin-induced renal damage

5. CONCLUSION

The aqueous seed extract of *Parkia clappertoniana* has curative but no nephroprotective effect on gentamicin-induced renal damage in Sprague-Dawley rats.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by Committee on Animal Research, Publication and Ethics (CARPE) of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana; Ethics Reference №: FPPS/PCOL/0022/2012.Laboratory study was carried out in a level 2 biosafety laboratory. All the technical team observed all institutional biosafety guidelines for protection of personnel and laboratory.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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