Cassia occidentalis Linn, Ageratum conyzoides Linn and Newbouldia laevis attenuate histoarchitectural dysfunction and biochemical derangement in gentamicin-induced nephrotoxicity in Wistar rats

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Nephrotoxicity is a major concern in the use of the antibiotic gentamicin. This study investigated the effect of aqueous leaves extract of Cassia occidentalis (CO), Ageratum conyzoides (AC) and Newbouldia laevis (NL) on histoarchitectue and biochemistry of Wistar rats in gentamicin-induced nephrotoxicity. Twenty male rats weighing 180 - 200g were used for the study. Gentamicin (100mg/kg) was co-administered with CO, AC and NL (100mg/kg or 150mg/kg) extract to rats in group 3 and 4. Rats in group 1 and 5 received distilled water and the extract, respectively. Group 2 rats received gentamicin, and served as positive control. Gentamicin was given interperitoneally once daily for 10 days, while the extract was given twice daily for 14 day, orally. Blood samples were collected by cardiac puncture for the analysis of serum creatinine, urea and electrolytes. The kidneys were excised for histopathological analysis. Serum creatinine and urea were 1.8mg/dL and 38.7mg/dL in group 2 and 0.8mg/dL and 19.7mg/dL for group 1 animals, respectively. The serum creatinine and urea in group 3 and 4 animals were significantly lower (p<0.05) compare to the positive control. Potassium ion was elevated by 2-fold in group 2, 3 and 4, relative to group 1. Histopathological analysis showed severe inflammation and tubular necrosis in the positive control, but not in the groups that received gentamicin and the extract. Cassia occidentalis, Ageratum conyzoides and Newbouldia laevis that attenuated gentamicin-induced nephrotoxicity in rats by preventing derangement of urea and creatinine and renal tubular necrosis is a potential source of nephro-protecting agent.
INTRODUCTION:
Gentamicin is a broad spectrum, effective and readily available antibiotic widely used for the treatment of Gram-negative bacterial infections. [1] Gentamicin exhibits dose-dependent and time-dependent kill a situation which most often than not result in the use of high doses for durations that might lead to undesirable side effects including irreversible renal toxicity. [1][2] The kidney as an important organ for the elimination of many xenobiotics including drugs, environmental chemicals, and metals is constantly at risk drug-induced toxicity and damage. [2] Renal toxicity related to drug use accounts for about 20% of the cases that may lead to acute kidney injury. [3]

Natural products especially plants have continue to play vital role as sources of drugs that are used clinically for the treatment of various diseases. [4] Some plants have also been reported to ameliorate drug-induced hepatotoxicity. [1] Notable examples are protective effect of Trigonella foenum-graecum against deltamethrin-induced haematological toxicity, [5] Terminalia muelleri in carbon tetrachloride-induced hepato and nephrotoxicity, [6] Stevia rebaudiana and Spirulina platensis against gentamicin-induced nephrotoxicity. [7, 1] Cassia occidentalis Linn, Ageratum conyzoides linn and Newbouldia laevis that are used for the treatment of various diseases in alternative and complementary medicine may also have potentials to attenuate drug-induce nephrotoxicity.

Cassia occidentalis, an herb that belongs to the family leguminosae, is commonly found in the southern, middle belt and northern parts of Nigeria. It is widely used for the treatment of many ailments including diarrhea, type 2 diabetes, dysentery, malaria, pains, typhoid, and leprosy. [8] Other ethnobotanical uses of the plant include; purgative, expectorant and diuretic. [9] Ageratum conyzoides Linn (Family: Asteraceae) is an annual herb with a long history use in traditional medicinal across the tropical and sub-tropical region of the world. [10] The herb is rich in sterols, flavonoids, and has biological activities such as free radical scavenging, anti-inflammatory and anticancer activities of the flavonoids. [10] Newbouldia laevis (P. Beauv) is a medicinal plant that belongs to Bignoniacae family. It is native to Guinea Savannah to dense forests of Nigeria and some other African countries. [11] Leaf extract of Newbouldia laevis contain divers phytochemicals such as alkaloids, flavonoids steroids, saponins, glycosides terpenoids and tannins vitaminic A, C and E. [11] The pharmacological activities of Newbouldia laevis range from laxative, anti-bacterial, anti-cancer to antisnake venom. [12]

Several studies have demonstrated the pharmacologic effects of Cassia occidentalis (CO), Ageratum conyzoides (AC) and Newbouldia laevis (NL). [8, 10, 12] However, there is no information on the effect of these plants on drug-induced nephrotoxicity. The aim of this research was to investigate the attenuating effects of leaves extract of CO, AC and NL on the histoarchitechture and biochemistry of Wistar rats in gentamicin-induced nephrotoxicity.

MATERIALS AND METHODS
Extract preparation
Plant samples of leaves of CO, AC and NL were collected washed, shade-dried and pulverized using Lexus mixer grinder. The decoction method as described by Lalrinzuali et al. [13] was employed for the extraction. Briefly, 10 g of the pulverized leaves of each plant was weighed and transferred into a beaker containing 200 ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 60°-80°C for 30 minutes. The aqueous extract was filtered and the filtrate evaporated to dryness on a water bath, packaged and stored in refrigerator at 4°C until used.

Experimental protocol
A total of twenty male Wistar rats (n=20) weighing 150 to 200 grams were procured form the Animal House, College of Health Science, Benue State University, Makurdi. The rats were randomly divided into five groups (n=4). Group 1 and 2 received only distilled water and 100mg/kg dose of gentamicin served as the negative and positive controls, respectively. Gentamicin was co-administered with
graded doses of the extract (100mg/kg or 150mg/kg) to rats in group 3 and 4. Gentamicin was administered intraperitoneally for 10 days, while the extract was orally administered twice daily for 14 days. Group rats received only 100mg/kg extract twice daily for 14 days. All experimental procedures were carried out in agreement with the guidelines on animal experiment as prescribed by the Ethical Committee of the Department of Anatomy, College of Health Sciences, Benue State University, Makurdi.

Biochemical analysis
At the end of the experiment, the rats were sacrificed using chloroform anesthesia. The blood was collected through cardiac puncture into non-heparinized tubes, allowed to clot for about 2 h and thereafter centrifuged at 4,000 g for 10 min to recover the serum that was used to measure the concentrations of creatinine and urea using Randox® creatinine and urea test kits (Randox Laboratories, UK).

Histopathological assessment
The kidneys of the experimental rats were excised, fixed in 10% formal saline and used for histopathology assessment. Kidney tissue samples were cut, embedded in paraffin and then sectioned into 5-6μm with a rotary microtome and stained using hematoxylin and eosin (H&E). The sectioned kidney tissues were assessed using a modification of the Banff criteria and scored for the presence or absence of glomerular changes, tubular interstitial atrophy, necrosis, inflammation or vascular changes such as interstitial fibrosis, hyalinosis or lumen occlusion. [14]

Statistical analysis
Data collected were expressed as the mean ± SD. Statistical significant difference was determined by one way analysis of variance (ANOVA) using the statistical package for social science (SPSS version 6) followed by Turkey’s post hoc test for multiple comparison. Level of statistical significance, α= 0.05.

RESULTS
Effect of aqueous leaves extract of CO, AC and NL on serum urea and serum creatinine
The effect of gentamicin and extract on serum urea is shown in figure 1. The serum urea level in gentamicin only treated group 2 was 38.7mg/dL, and significantly higher (p<0.05) compared to 19.7mg/dL in the group of rats that served as the negative control. The levels of serum urea in group 3 and 4 rats in which graded doses of the extract was co-administered with gentamicin were significantly lower (p<0.05) compared to serum urea of rats in group 2. There was no statistical difference (p>0.05) in serum levels of urea of group 1 and 5 rats that received distilled water and the extract only (figure 1).

The serum creatinine of Wistar rats in gentamicin-induced hepatotoxicity treated with CO, AC and NL extract is presented in figure 2. The administration of gentamicin for ten days significantly increased (p<0.05) the serum creatinine (1.8mg/dL) in the positive control (group 2) rats compared to 0.5 mg/dL in the negative control (group 1). However, serum creatine in groups that received both gentamicin and the extract at 100mg/kg and 150mg/kg was 1.28 and 0.84 mg/dL, respectively. This was significantly lower...
(p<0.05) compared to 1.8mg/dL in the gentamicin only group. The serum creatinine of rats that received only distilled water or the extract were not significantly different (p>0.05).

**Effect of aqueous leaves extract of CO, AC and NL on plasma electrolytes**

Figure 3 shows the plasma sodium and chloride ions of Wistar rats in gentamicin-induced hepatotoxicity. There was no significant differences (p>0.05) in plasma sodium ion and chloride ions observed between gentamicin treated rats the group of rats, gentamicin co-administered with leaves extract of CO, AC and NL or negative control (Figure 3).

As shown in figure 4, the levels of serum potassium in the group of rats treated with gentamicin (group 2) was significantly higher (p<0.05) compared to the negative control, group 1 that was not treated with gentamicin. However, the level of potassium ion in the group of rats in which gentamicin was co-administered with the extract (100mg/kg or 150 mg/kg) was not significantly different (p>0.05) compared to the positive control (Figure 4).
Histological analysis

The histological analysis of the kidneys excised from rats in which gentamicin was used to induce hepatotoxicity are presented in figure 5a-e. Severe glomerular and tubular necrosis at the proximal section, interstitial inflammation and fibrosis of the stroma and sloughing of epithelia cells with occlusion of tubular lumens were observed in the kidneys of rats in group 2 that received gentamicin only (Figure 5b). However, the kidneys of rats that received both gentamicin and graded doses of the extract at 100mg/kg or 150mg/kg did not show abnormal glomerular, stroma damages, tubular atrophy or tubular necrosis (Figure 5c and d). The negative control group, group 1 and the group 5 that received only distilled water or extract, respectively showed normal kidney histology with no structural damages (Figures 5a and e).

Fig. 5: (a-e) Photomicrographs of the sectioned rats kidney (H & E x 40). (a and e) Normal histology of kidney tissue in negative control and extract treated rats. (b) Kidney tissue of gentamicin-induced hepatotoxicity showing tubular damage and necrosis (arrow) and inflammatory cells (circle). Sectioned kidney of rats treated with gentamicin co-administered with 100 mg/kg (c) 150mg/kg (d) of extract.
DISCUSSION

Nephrotoxicity is one of the one major complications of gentamicin that limits the optimal clinical use of this cheap, available and effective antibiotic that is often used alone or in combination with other antibiotics for the treatment of severe gram-negative bacterial infections. \[15\] Gentamicin-induced nephrotoxicity model in rodent that is regarded as the ‘gold standard’ for kidney-related toxicity studies was employed in this research. \[16\] *Spirulina platensis, Trigonella foenum-graecum, Terminalia muelle, Stevia rebaudiana* and some chemical molecules have been reported to reduce drug-induced hepatic or nephrotoxicity in rats. \[5, 6, 7, 1\] In this study, the effect of aqueous leaves extract of *Cassia occidentalis* (CO), *Ageratum conyzoides* (AC) and *Newbouldia laevis* (NL) on gentamicin-induced nephrotoxicity was investigated.

Creatinine is one of the biomarkers used for the clinical assessment of renal function and glomerular filtration rate. \[15, 3\] Creatinine undergoes glomerular filtration and, to some extent tubular secretion. An elevated serum creatinine is an indication of decreased glomerular filtration and renal failure. \[15, 17, 18\] The results from this study show that 10 days administration of gentamicin at a dose of 100mg/kg to rats produced nephrotoxicity that was evidenced by a 3.6 fold elevation in the serum creatinine. The results from this study partly agree with findings of Udupa and Prakash \[15\] who reported a 3.4 fold increase in serum creatinine on day 10 of administration of 100mg/kg of gentamicin, respectively, relative to the control.

Serum urea is a metabolic waste product that is excreted by the kidneys in urine. \[15\] Urea is filtered from the blood to the glomerular filtrate, and excreted in the urine. In renal failure, there is decreased filtration of urea into urine, a condition that leads to elevated serum urea. \[19, 15\] In this study there was a 2 fold increase in the serum urea relative to the negative control. The result from this study slightly defer from the 5.6 fold increase in serum urea reported by Udupa and his collaborator \[15\] in a 10 day administration of 100mg/kg/day of gentamicin to Wistar rats. The differences in the number of fold may be related to method used for evaluation of urea. \[15\]

Increase in serum creatinine and serum urea was associated with severe glomerular changes, renal tubular necrosis and marked presence of inflammatory cells infiltration indicating decline in kidney function. Observations by other researcher have also reported renal tubular degeneration and necrosis and infiltration of inflammatory cells with gentamicin at 100mg/kg/day on day 8. \[19, 15\] This suggests that the daily administration of 100mg/kg/day of gentamicin caused structural renal damage and alteration of renal functional.

In this study, the co-administration of extract of CO, AC and NL with gentamicin showed significant decrease in the levels of serum creatinine and urea relative to the positive control. The decrease in these two biomarkers used in the diagnosis of renal failure by the extract correlate with protection against gentamicin-induced glomerular and renal tubular damage. This suggests that the extract of CO, AC and NL might have interfered with the process by which gentamicin induces nephrotoxicity in Wistar rats thereby attenuating such effect.

It has been hypothesized that gentamicin accumulates in cells of the renal proximal tubule at concentration that is several times higher than in plasma during the process of excretion. \[19\] This results in the generation of reactive oxygen species (ROS) that stimulate the activation of proinflammatory mediators, including NF-κB, leukocyte adhesion molecules, and mitogen-activated protein kinases (MAPKs) that mediate the progressive gentamicin-induced kidney damage. \[20, 7, 21\] Other mechanisms by which reactive oxygen species produce cellular injury and necrosis includes lipoperoxidation and protein modification. \[7\]

In the light of the foregoing, the protective effect of the extract is proposed to be by mechanisms involving either increased excretion of gentamicin or decreased tubular uptake and accumulation of gentamicin that mediate gentamicin-induced nephrotoxicity or free radical scavenging effect. Notably, leaves extracts of CO, AC and NL are rich in phenolics such as flavonoids and tannins that have been shown to exhibit excellent free radical scavenging activity. \[122, 10, 11\]

Although *in vivo* or *in vitro* antioxidant assays were
out the scope of this study, it would be plausible to suggest that the probable mechanism of renal protection by leaves extracts of CO, AC and NL is its free radical scavenging activity.

Gentamicin-induced impairment of the renal function is suggested to involve the activation of the renin-angiotensin system that consequently leads to ROS-induced local vasoconstriction with a resultant decrease in glomerular filtration. [23] This hypothesis is strengthened by the fact that nonsteroidal anti-inflammatory drugs that inhibit the production of the vasodilating prostaglandin PGE2 have been found to exacerbate aminoglycoside nephrotoxicity. [24] The above information further explains why antioxidants, and leaves extract of AC, CO and NL are able to attenuate gentamicin-induced glomerular and tubular dysfunction. It has been predicted that gentamicin nephrotoxicity is caused by tubular cytotoxicity by an apoptotic pathway that is related to the release of lysosomes. [21] Gentamicin is known to accumulate in renal proximal tubules and then transported into lysosomes. Gentamicin binds to lysosomal phospholipids resulting in gentamicin-induced phospholipidosis that is directly related to tubular necrosis and apoptosis observed in gentamicin-induced nephrotoxicity. [25, 21] The alteration in electrolyte transport is as a result of the tubular necrosis and cellular dysfunction caused by the lysosomal enzymes. [26, 21]

In this study, serum sodium and chloride ions were unchanged with the administration of gentamicin. However, potassium ion was increase in gentamicin treated rats, but not ameliorated with co-administration of leaves extract of AC, CO and NL. Conflicting results have been reported with respect to changes in tissue potassium after long-term gentamicin administrations. [27] Tissue potassium which consequently affects the serum potassium has been reported by some researchers to increase, [28] to decrease [29] or unchanged [30] with the administration of gentamicin. The changes in the transmembrane fluxes of electrolytes may be driven by the direct action of gentamicin on proximal tubule cells. [27] This is because of the convincing evidence demonstrating that gentamicin impairs Na-K/ATPase activity of renal tubular cells and increases membrane sodium. [31, 30] The rise in serum potassium concentration observed in this study could be due to increased potassium uptake via the Na-K/ATPase or some unknown mechanism.

**CONCLUSION**

Gentamicin-induced nephrotoxicity is a limiting factor to optimizing the clinical benefits of this drug in antimicrobial chemotherapy. *Cassia occidentalis*, *Newboulda laevis* and *Ageratum conyzoides* when co-administered with gentamicin attenuated gentamicin-induced nephrotoxicity evidenced by the significant reduction in serum creatinine and urea, prevention of alteration of renal structural architecture. *Cassia occidentalis*, *Newboulda laevis* and *Ageratum conyzoides* would likely increase gentamicin safety and serve as a promising source of novel plant-derived nephroprotective agent in drug-induced nephrotoxicity.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Authors’ Declaration**

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

**REFERENCES**


