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Research article

Protective effect of aqueous leaf extract of *vernonia amygdalina* (bitter leaf) on gentamicin induced nephrotoxicity in adult wistar rat

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ABSTRACT

This study evaluated the protective effect of an aqueous leaf extract of *Vernonia amygdalina* on the kidney functions and histological architecture of gentamicin-induced nephrotoxicity in adult Wistar rats. Twenty-five (25) Wistar rats were divided into five groups (groups 1–5), with five rats in each group. Group 1 served as the normal control. Group 2 was the negative control and received 40 mg/kg of body weight of gentamicin. Groups 3, 4, and 5 received an aqueous extract of *V. amygdalina* at doses of 100 mg, 200 mg, and 400 mg/kg of body weight, respectively, for 21 days. The gentamicin was administered intraperitoneally for 14 days, starting on day 8, while the aqueous leaf extract was given orally. There was a significant ($P< 0.05$) increase or derangement in the serum levels of urea, creatinine, and potassium in group 2 when compared to the normal control. The groups that received the extract of *V. amygdalina* showed a significant ($P<0.05$) reduction in the levels of deranged serum urea, creatinine, and potassium compared to group 2. The kidney histological photomicrograph of group 2 was characterized by epitheloid granulomas, glomerular necrosis, and severe nephrotic tissue degeneration. The groups treated with the extract showed significant protection against these alterations. The protection was found to be dose-dependent. It was discovered that *V. amygdalina* had a dose-dependent protective effect against kidney damage caused by gentamycin toxicity.

Keywords: Renal protection, Gentamycin, *Vernonia amygdalina*, Biological salts, Extracts.

INTRODUCTION

The kidneys are responsible for the excretion of toxic metabolites, extracellular fluid control, and homeostasis [1]. Damages and diseases of the kidneys impair these functions, thereby leading to fluid retention and the accumulation of toxic products [2]. Diseases of the kidney are common and cause huge financial burdens to the patient or family and the country at large, particularly in developing countries like Nigeria [3]. There are various forms of kidney diseases, such as nephrotic syndrome, acute interstitial nephritis, acute glomerulonephritis, polycystic kidney diseases (PKDs), and acute and chronic renal failures. Some of these can be congenital (e.g., PKDs) or secondary to other disease conditions such as diabetes mellitus, hypertension, severe hemorrhages, and infections. However, about 20% of kidney diseases are said to be caused by drugs or chemicals, especially anticancer medications and other chemotherapeutic agents [4]. The accumulation of gentamicin in the cortex of the kidney can cause kidney disease, which can account for a significant percentage of kidney toxicities [5].

Gentamicin is an antibiotic of the aminoglycoside group widely used against susceptible gram-negative microorganisms [6]. It has been used against bacterial strains that are resistant to other antibiotics in a variety of conditions, including urinary tract infections and endocarditis. However, its use is limited due to adverse effects such as kidney and liver toxicity [7]. Kidney damage from gentamycin usually shows tubular necrosis, especially in the proximal tubule [6,8]. This effect is probably due to gentamycin accumulation in the proximal convoluted tubules. The major mechanism of gentamicin-induced kidney damage was said to be the formation of free radicals [4].

Many plant extracts possess antioxidant properties and have been reported to be effective in protecting some organs from toxicities. These therapeutic potentials are exhibited with little or no adverse effects [9]. The antioxidant effect of *V. amygdalina* is probably due to its flavonoid content [10]. *Vernonia amygdalina* belongs to the Asteraceae family and grows widely in Africa. Its name was derived from the name of an English botanist, "William Vernon" [11]. but it is known as bitter leaf because it has a bitter taste. It is said to be rich in fats, proteins, fibers, minerals, amino acids, carbohydrates, and vitamins [11,12]. The leaf extracts also contain flavonoids, polyphenols, saponins, tannins, and terpenoids [13]. These phytochemicals might be responsible for their anti-diabetic, antimicrobial, anti-allergic, anti-cancer, anti-inflammatory, and anti-oxidative effects [14,15]. As a result, it is extremely beneficial in traditional or herbal medicine [16, 17].

This study was aimed at evaluating the protective effect of an aqueous leaf extract of *V. amygdalina* on the kidney functions and histological architecture of gentamicin-induced nephrotoxicity in adult Wistar rats.

MATERIALS AND METHODS

Collection and authentication of plant

The leaves of *V. amygdalina* were obtained from a natural habitat in Aninri Local Government Area of Enugu State. The leaves were authenticated at the Botany Department, University of Nigeria, Nsukka Campus, with voucher number UNH351.

Preparation of extracts

The fresh leaves were washed very well with tap water and allowed to dry under shade at room temperature for a period of 7 days. The dried leaves were ground into a uniform powder using a household blender and sieved with a 60-mesh sieve to obtain a fine powdered particle, which was put in an airtight container. Thereafter, the powdered extract was thoroughly mixed with distilled water and allowed to stand for 72 hours. The mixture was sieved with cheesecloth and further filtered with Whatman's filter paper size 1 to obtain a clear filtrate of the extract. The filtrate was concentrated in a water bath at 80 °C to obtain the concentrate (extract). This weighs 120.5 grams. The percentage yield was 7.09%. An airtight container was used to store the extract, which was kept in a refrigerator at 4 °C until use.

Procurement of drugs and chemicals

Gentamicin (manufactured by Abbott Healthcare Pvt. Ltd.) was obtained from a reputable pharmacy shop in Enugu. Formaldehyde (40%) was purchased from a chemical and reagent dealer at Ogbete Main Market in Enugu State.

Experimental animals

Twenty-five (25) Wistar rats (weighing 180–200 g) were obtained at the animal house in the College of Medicine, University of Nigeria, Enugu Campus (UNEC). They were kept in cages and were provided with easy access to food (a Chukun finisher) and water ad libitum. The animals were maintained under 12-hour light and dark cycles and were allowed to acclimatize for fourteen days prior to the experiment.

Experimental design

After acclimatization, they were grouped into 5 groups of 5 rats each.

Group 1(the normal control) received feed, distilled water, and then normal saline for 21 days.

Group 2(the negative control) received gentamicin (40 mg/kg) for 21 days with no extract.

Group 3 received *V. amygdalina* extract at 100 mg/kg for 7 days, followed by the extract at 100 mg/kg body weight and gentamicin (40 mg/kg) for 14 days.

Group 4 received a *V. amygdalina* extract (200 mg/kg) for 7 days, followed by a *V. amygdalina* extract (200 mg/kg body weight plus gentamicin (40 mg/kg) for 14 days.

Group 5 received 400 mg/kg body weight of the extract for 7 days, and then the extract at 400 mg/kg body weight and 40 mg/kg body weight of gentamicin for 14 days.

The gentamicin was administered intraperitonially, and the extract was given orally.

Sample collection and sacrifice of animals

Twenty-four hours after the last administration, blood samples were collected from the rats through the retro-orbital plexus for biochemical studies. Thereafter, the rats were sacrificed using chloroform as an anesthetic. The harvesting of the kidneys was done by making a mild incision through the abdominal wall. For histological examination, the harvested kidneys were fixed in 10% formol saline for 48 hours.

Biochemical analysis

The blood samples were taken to the laboratory for quantitative studies. The serum urea nitrogen, creatinine, and potassium levels were measured, and the values were recorded.

Histological examination

The tissues were processed for microscopic examination using standard protocol [18].The fixed kidneys were dehydrated in different grades of alcohol (70–95%). Clearing was done through 2 changes of xylene for 2 hours each, and infiltrating with 2 changes of paraffin wax for 2 hours. The sections were cut at 5 mm using a rotary microtome. Staining was done with hematoxylin and eosin.

Ethical approval

The Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus, gave its ethical approval for the study procedure.

Data analysis

This was done using the Statistical Package for Social Science (SPSS), version 23. The results were displayed as the mean and standard deviation. A one-way ANOVA was used to compare the results between the various groups, and the significant level was set at $p< 0.05$.

RESULTSAND DISCUSSION

This study was aimed at evaluating the effect of *V. amygdalina* on the kidney functions and histological architecture of gentamicin-induced nephrotoxicity in adult Wistar rats. The gentamicin caused damage to the kidneys, as evidenced by the significant and abnormal elevation of the serum urea, creatinine, and potassium levels (79.00±7.07 mg/dl, 0.85±0.07 mg/dl, and 7.15±0.49 mg/dl, respectively) (table 1), as well as epithelioid granulomas, necrosis of the glomeruli, and severe nephrotic tissue degeneration seen in the histology photomicrograph of the kidneys (figure 2). These toxic effects of gentamicin on the kidney were probably due to the inhibition of protein synthesis in the cells of the renal tubules, resulting in acute tubular necrosis. Our result is consistent with the findings of other studies, which showed that gentamicin caused renal damage with derangements in blood creatinine, urea, and potassium levels in patients and animal models [19-21]. It has been reported that gentamicin treatments cause kidney damage, which manifests as a decrease in renal function, as evidenced by an increase in serum creatinine and urea levels, as well as impairment in glomerular functions [8]. Other researchers noted similar findings [22,23]. They found that gentamicin caused increased creatinine and urea levels. It was stated that exposure to gentamicin resulted in progressive glomerular, tubular, and interstitial histological alterations [24].

It was shown in this study that *V. amygdalina* gave significant protection to the renal functions by gradually restoring the biochemical parameters to normal values (table 1), especially in the high dose group (low dose: urea, 64.50±0.71; creatinine, 0.65±0.07; potassium, 6.65±0.64; medium dose: urea, 56.50±2.12; creatinine, 0.55±0.07 potassium, 5.35±0.21, and high dose: urea, 40.50±2.12; creatinine, 0.30±0.14 potassium, 4.30±0.14). It also gave significant protection to the histological tissue architecture in all the treated groups (Figures 3, 4, and 5). However, the protection is dose-dependent, with the highest dose providing the best protection (Figure 5), and the renal function parameters returned to near normal (Table 1). Though the histological photomicrograph still showed mild damage, the presence of mild damage noted (even when the renal functions have returned to normal) was because the biochemical parameters recovered faster than the tissue damage. Generally, this protective effect of *V. amygdalina* is likely related to the flavonoid content, as reported by other researchers [10,25,26].This supports the findings of other authors, who stated that *V. amygdalina's* renal tissue recovery potential is due to its high antioxidant defense capacities from flavonoids, alkaloids, and polyphenols [16]. *Vernonia amygdalina* has been shown to promote wound healing via its antioxidant flavonoid effect [27].

Biochemical Results

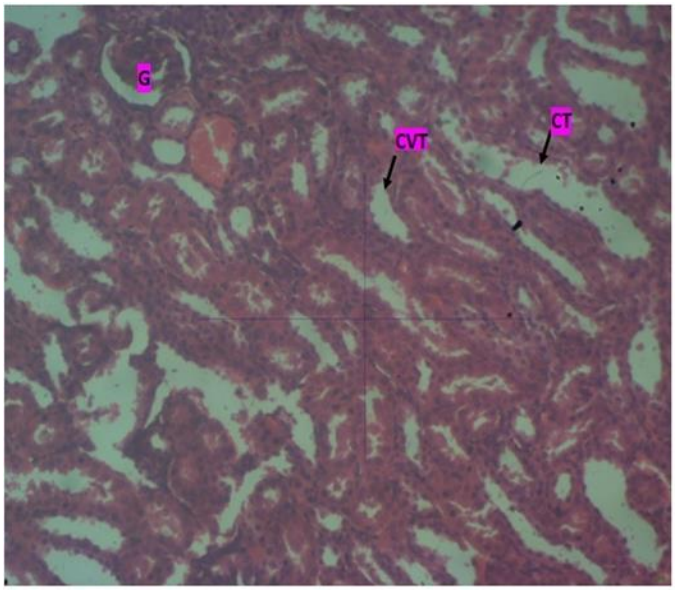
Table 1: Mean ± SD of the urea, creatinine, and potassium in the different groups

Animal Groups	Urea (mg/dL)	Creatinine (mg/dL)	Potassium (meg/L)
	M±SD	M±SD	M±SD
Normal control	37.50±2.12	0.30±0.00^{a}	4.70±0.28^{ab}
Negative control (Gentamicin on	79.00±7.07	0.85±0.07^{c}	7.15±0.49^{d}
Gentamicin/extract low dose	64.50±0.71	0.65±0.07^{b}	6.65±0.64^{d}
Gentamicin/extract medium dose	56.50±2.12^{b}	0.55±0.07^{b}	5.35±0.21^{bc}
Gentamicin/extract high dose	40.50±2.12	0.30±0.14^{a}	4.30±0.14^{a}
P-Value	< .001	0.001	0.001

Means in the same column with different superscript letter(s) differ significantly ($p < 0.05$).

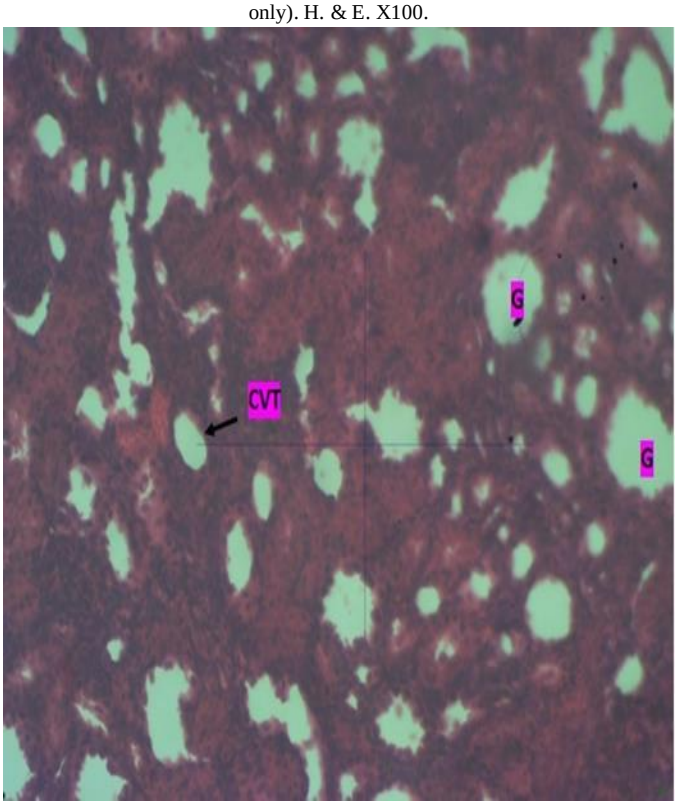
Histological Results

Figure 1: Photomicrograph of the kidney of rats in Group 1 (control). H. & E. X100.



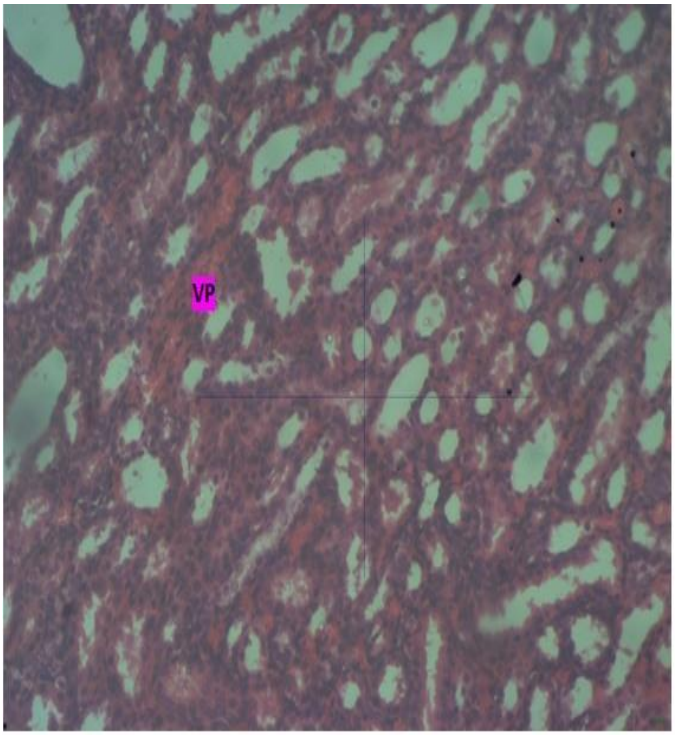
G-Glomerulus, CVT-convoluted tubules, and CT-collecting tubules showed no obvious pathology.

Figure 2: Photomicrograph of the kidney of rats in Group 2 (gentamycin only). H. & E. X100.



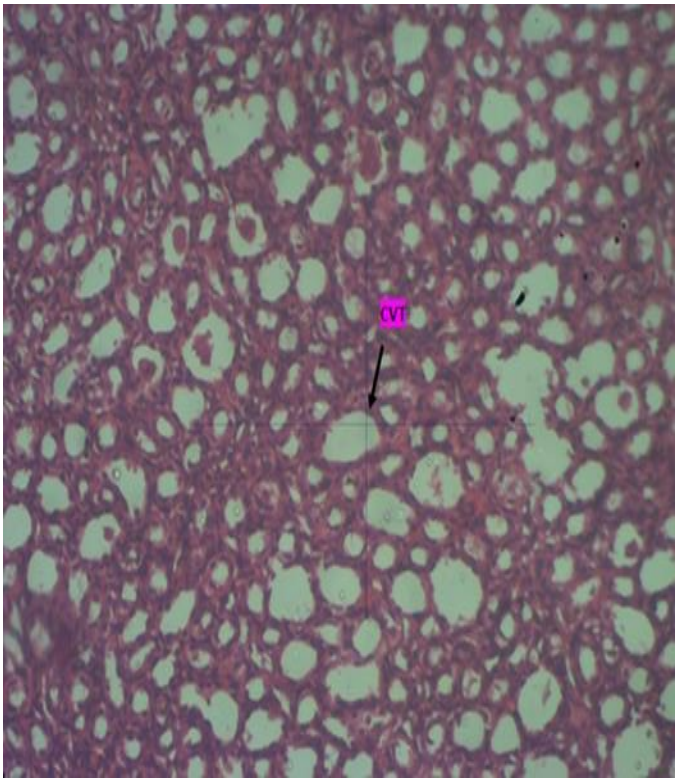
G-Glomerulus, CVT-Convoluted Tubules. (There is pigmented tissue with convoluted tubules (Cvt) filled with epitheloid granulomas and glomerular necrosis (G).parenchyma shows severe nephrotic tissue degeneration).

Figure 3: Photomicrograph of the kidney of rats in Group 3 (gentamycin plus a low dose of extract). H. & E. X100.



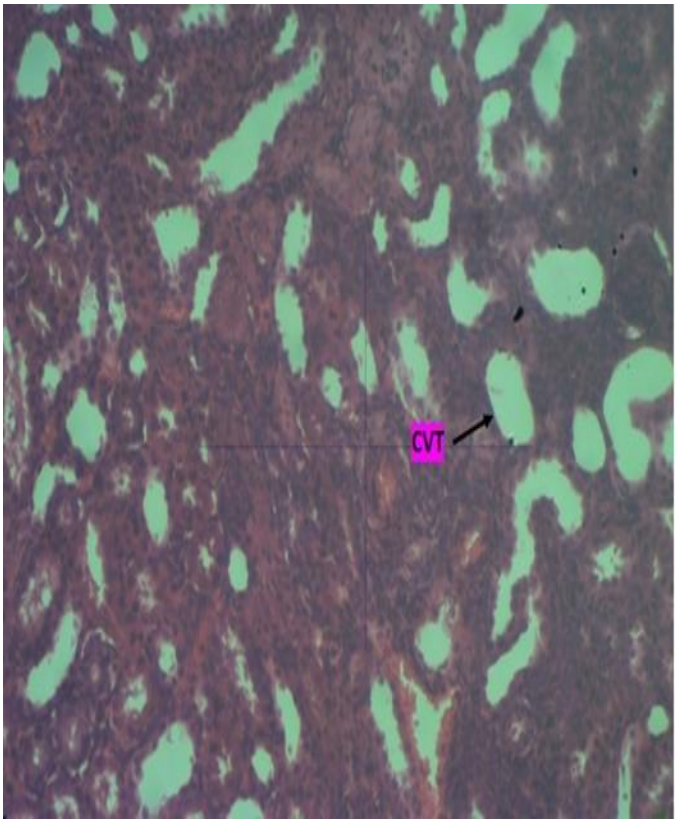
VP-vascular pigmentation. There is mild focal vascular pigmentation (Vp) and peripheral displacement of the epithelium of convoluted tubules (Cvt) with loss of brush borders.

Figure 4: Photomicrograph of the kidney of rats in Group 4 (gentamycin plus a medium dose of extract). H. & E. X100.



CVT-Convoluted Tubules. Parenchyma shows tubular vacuolations and peripheral displacement of epithelium in convoluted tubules (CVT).

Figure 5: Photomicrograph of the kidney of rats in group 5 (gentamycin plus a high dose of extract). H. & E. X100.



CVT-Convoluted Tubules. Tissue parenchyma shows a pigmented and hyalinized microstructure with mild vacuolations.

CONCLUSION

This study showed that the leaf extract of *Vernonia amygdalina* had a protective effect on both the renal biochemical parameters and renal structural damage in rats exposed to gentamycin nephrotoxicity. The protection was evident in groups that received both low and medium doses but was more pronounced with the high dose of the extract. Thus, *V. amygdalina* may be beneficial as a herbal remedy for kidney diseases.

Conflict of interest

There is no conflict of interest.

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