Kolaviron alleviates haematological abnormalities and hepato-renal damage in *Naja nigricollis nigricollis* venom-treated rats

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**A R T I C L E  I N F O**

Handling Editor: Prof. L.H. Lash  

**Keywords:**  
Antioxidant  
Haematological abnormalities  
Hepato-renal damage  
Kolaviron  
NNN venom

**A B S T R A C T**

Objectives: To investigate the effect of kolaviron against haematological abnormalities and hepato-renal damage in *Naja n. nigricollis* (NNN) venom-treated rats.

Methods: Twenty-four male rats were grouped into four (*n* = 6). A single intravenous dose of NNN venom (≈2%LD₅₀) was given to group B-D (excluding A). All the groups were immediately treated intraperitoneally as follows: A (Normal control) and B (Envenom) received 0.40 mL/kg of 0.1% Tween 80, while C and D (test groups), received 200 and 400 mg/kg of kolaviron respectively. After 6 h, they were anesthetized, and sacrificed. Results: NNN-venom LD₅₀ was estimated at 1.14 mg/kg. Injected half LD₅₀, after 6 h, caused significant (p < 0.05) decreases in RBC, HGB and PCV, with increases in WBC and NEUT. Significantly (p < 0.05) increased AST, ALT, GGT, TB, CRE, URE, UA and K with concomitant decreases in Na and HCO₃⁻. Oxidant/antioxidant markers (MDA, CAT and SOD) were significantly (p < 0.05) increased in liver and kidney homogenates. Histological analysis confirmed liver and kidney injuries. All these alterations were alleviated dose-dependently, when cotreated with kolaviron at 200 and 400 mg/kg.

Conclusions: Our study suggests that kolaviron could alleviates haematological abnormalities and hepato-renal damage in NNN venom-treated rats by depleting ROS and/or boosting the antioxidant system.

**1. Introduction**

Snakebite envenoming remains a major public health concern especially in the rural areas of tropical countries like Nigeria [1]. *Naja nigricollis nigricollis* (NNN), belonging to Elapidae family, is one of most dangerous snakes responsible for serious medical emergencies in Nigeria [2,3]. *Naja n. nigricollis* snake dominates the rainforest of South-eastern Nigeria, probably because of its unique adaptive features and defensive behavior [4,5].

The venom of *Naja n. nigricollis* comprises mainly neurotoxins, cytotoxins (a three finger toxins), phospholipase A₂ (PLA₂), hyaluronidase (HYA) and peptides [6]. Local tissue injuries are more common after bites from many *Naja* species including *Naja n. nigricollis* [7]. This is usually thought to be due to the action of cytotoxins (CTX) and PLA₂ [8], which disrupt the plasma membrane of different cells and induce oxidative stress via ROS formation [9] leading to cellular damage such as haematological abnormalities [7,9], nephrotoxicity [10], and hepatotoxicity [11] etc. These effects, in many cases, cannot be neutralized even by anti-snake venom [12], but their severity could be reduced by the use of natural and/or synthetic antioxidant compounds [13].

Previous studies have shown that some plant-based flavonoids can alleviate this venom induced complications [14,15]. Kolaviron (a biflavonoid) from *Garcinia kola* is a well-known powerful antioxidant compound [16] with immense therapeutic properties e.g., anti-inflammatory [17], anti-jaundice [1,8], renoprotective [19-21] and hepatoprotective properties [22] etc. Based on these facts, the present study attempts to investigate the possible effect of kolaviron (KV) against haematological abnormalities and hepato-renal damage caused by *Naja n. nigricollis* venom treatment in rats.

**Abbreviations:** NNN, *Naja nigricollis nigricollis*; ROS, Reactive oxygen species; PLA₂, Phospholipase A₂; LD₅₀, Median Lethal dose; CTX, Cytotoxins; HYA, Hyaluronidase; KV, Kolaviron.

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**https://doi.org/10.1016/j.toxrep.2022.10.003**

Received 21 June 2022; Received in revised form 29 July 2022; Accepted 3 October 2022

Available online 4 October 2022

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2. Materials and methods

2.1. Chemicals and reagents

Glacial acetic acid (CH₃COOH) and potassium heptaoxodichromate (K₂Cr₂O₇) were purchased from Sigma Aldrich Chemical (St Louis, MO, USA). Trichloroacetic acid (TCA) and disodium hydrogen orthophosphate (Na₂HPO₄) were purchased from BDH Company, U.K. Sodium dihydrogen phosphate (NaH₂PO₄) from May and Baker Ltd England. Sodium hydroxide (NaOH), 2-thiobarbituric acid (TBA), hydrochloric acid (HCl), formaldehyde (CH₂O) and tween 80 are from Loba chemie Ltd. India. Ketamine hydrochloride injection USP from Swiss Parenterals Pvt. Ltd. Gujarat, India. All other chemicals used were of analytical grade.

2.2. Venom sample

Naja n. nigricollis venom was kindly provided by Dr. Peter Ofili, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The lyophilized snake venom sample was stored at 4°C. Stock solutions of venom were reconstituted in saline solution (0.9% NaCl) and kept frozen at −20°C prior to use.

2.3. Test sample

Kolaviron was obtained from Division of Pharmacology, Central Drug Research Institute (CDRI), Lucknow, India and authenticated by Liquid Chromatography-Mass Spectrometry (LC-MS) analysis [1]. Kolaviron was further dissolved in 0.1% Tween 80 and considered as test sample.

2.4. Kolaviron chromatogram

Kolaviron was authenticated using Liquid Chromatography-Mass Spectrometry (Waters Corporation, Milford, Massachusetts, USA) as described by Okafor and Onyike [1]. Kolaviron was subjected to reversed phase chromatography on Acquity BEH C18 (1.7 µm, 2.1 × 50 m) Ultra Performance Liquid Chromatography (UPLC) column using gradient elution with 0.1% formic acid in water (solvent A) and Methanol/Acetonitrile (1:1; solvent B) at a flow rate of 1.0 mL/min for 20 min. MS spectra data were acquired in positive mode using Xevo TQ Detector scan wave collision cell.

2.5. Experimental animals

Male albino Wistar rats weighing 180–220 g were procured from Animal Farm, Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH), Okuku Campus, Nigeria. They were housed at a room temperature of 25°C with 12-h light/dark cycle, good ventilation, and unlimited access to standard diet (Vital feed, Jos) and water. Ethical approval for this study (CRUTECH/FBMS/IREC/2021-A125) was obtained from CRUTECH Institutional Research and Ethical Committee (IREC). All procedures were performed in compliance with IREC guidelines, followed by National Research Council’s Guide for the Care and Use of Laboratory Animals.

2.6. Determination of Naja n. nigricollis venom lethality in rats

The median lethal dose (LD₅₀) of Naja n. nigricollis venom was determined in rats according to the method of Meier and Theakston [23]. Various doses (i.e., 0.30, 1.30, 2.30, and 3.30 mg/kg) of venom in 0.2 mL of saline were injected intravenously into rats weighing 180–220 g (n = 10). Deaths occurring within 24 h post envenomation were used to construct graphic values for determination of Naja n. nigricollis venom LD₅₀ in rat [24]. An approximate estimate of LD₅₀ was calculated from a plot of probits vs. Log concentration at 50% confidence limit [25].

2.7. Experimental grouping and treatment

Twenty-four (24) male albino Wistar rats (180–220 g) were assigned to four Groups (n = 6). A single intravenous dose of NNN venom (approximately ½ LD₅₀) was given to group B to D (excluding Group A). All the groups were immediately treated intraperitoneally as follows: Group A (Normal control) and Group B (Envenom) received 0.40 mL/kg of 0.1% Tween 80, while C and D (Test groups), received 200 and 400 mg/kg of kolaviron respectively. At the end of experiment (i.e., 6 h post-envenomation), all the animals were anaesthetized intraperitoneally with ketamine (at 90 mg/kg) and sacrificed. The doses of venom (cum period) and kolaviron were selected based on pilot study.

2.8. Blood sample

For haematological assays, blood samples (via cardiac puncture) were collected in EDTA containers. The concentration of haemoglobin (HGB), packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and neutrophil (NEU) were analysed using an automated cell counter. In contrast, blood drawn into plain vacutainer tubes (without anticoagulants) were spun at 1500 x g for 10 min. Serum samples were harvested and used to monitor several biochemical markers of the liver and kidney. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin (TB), urea (URE), creatinine (CRE), and uric acid (UA) levels were determined using commercially available kits from Randox Lab, Antrim UK. While sodium (Na), potassium (P) and bicarbonate (HCO₃⁻) were assayed for serum electrolyte using Micropoint diagnostic test kit USA. All the kits assay were performed in accordance with the manufacturer’s recommended instructions.

2.9. Oxidant and antioxidant parameters

The liver and kidney samples were excised and blotted immediately after sacrifice. A portion of the samples were homogenized in a chilled saline at 5% w/v, using homogenizer. The homogenate was centrifuged at 3000 x g for 20 min, and the supernatant obtained was used for analysis. Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) formed as malondialdehyde, according to the method of Buege and Aust [26]. In addition, catalase and superoxide dismutase activities were evaluated according to the method of Sinha [27] and Martin et al. [28] respectively.

2.10. Histopathological analysis

Conventional method of paraffin-wax sectioning and haematoxylin-eosin staining were employed for histological studies [29]. Pieces of liver and kidney tissue were carefully removed and fixed in a 10% buffered formalin for 24 h. They were further dehydrated, embedded in a wax, sectioned into 5 μm thickness using a microtome, and then stained with haematoxylin and eosin. The stained sections were examined by Olympus light microscope (Olympus, Tokyo, Japan) with a high-resolution digital camera system.

2.11. Statistical analysis

The results were expressed as the mean ± standard deviation (S.D) and statistical analysis was performed using GraphPad Prism version 5.00 (GraphPad Software, La Jolla California USA). Differences among the groups were investigated using one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test. p < 0.05 was considered statistically significant.
3. Results

3.1. LC-MS analysis of Kolaviron

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis of kolaviron gave six (6) peaks in the positive mode (Fig. 1). Each of these peaks were analysed by electrospray ionization coupled with Mass spectrometer (ESI-MS) and presented as follows: (1) m/z 573.19, (2) m/z 557.28, (3) m/z 587.38, (4) m/z 557.26, (5) m/z 541.30 and (6) m/z 555.30 (Table 1). Based on the molecular formulae, the presence of Garcinia biflavonoid 2 (m/z 573.19 = C30H22O12), Garcinia biflavonoid 1 A & B (m/z 557.28, 557.26 = C30H22O11), kolaflavanone (m/z 587.38 = C31H24O12), binaringenin (m/z 541.30 = C30H22O10) and kolaflavones (m/z 555.30 = C30H21O11) were identified.

3.2. Median lethal dose (LD50) determination of NNN venom in rats

Median lethal dose (LD50) of NNN venom was assessed intravenously in rats. Most of the envenomed animals displayed signs of acute toxicity, including progressive malaise, lethargy, neurotoxicity (e.g., muscle trembling, paralysis or convulsions), coma and even death. After 24 h post envenomation, 20%, 50%, 70%, and 100% mortalities were recorded in group B to E respectively, which corresponds to different concentrations (0.30, 1.30, 2.30, and 3.30 mg/kg) of NNN venom (Table 2). Using probit curve, LD50 for NNN venom was estimated to be 1.14 mg/kg in rats (Fig. 2).

3.3. Kolaviron suppressed haematological abnormalities in NNN venom-treated rats

Table 3 showed the effect of KV on some haematological parameters (e.g., RBC, HGB, PCV, WBC and NEUT) in NNN envenomed rats. In the

| Peak Number | Retention Time (min) | Base Peak (m/z) | Molecular Formulae | Compound name |
|-------------|----------------------|----------------|--------------------|---------------|
| 1           | 8.655                | 573.19         | C30H22O12          | Garcinia biflavonoid 2 |
| 2           | 9.098                | 557.28         | C30H22O11          | Garcinia biflavonoid 1A |
| 3           | 9.206                | 587.38         | C31H24O12          | Kolaflavanone |
| 4           | 9.396                | 557.26         | C30H22O11          | Garcinia biflavonoid 1B |
| 5           | 9.917                | 541.30         | C30H22O10          | Binaringenin |
| 6           | 10.690               | 555.30         | C30H21O11          | Kolaflavones |

Fig. 1. Kolaviron chromatogram using Liquid Chromatography-Mass Spectrometry (LC-MS).
animals that received single dose of NNN venom at 0.57 mg/kg (≈1LD₅₀) after 6 h, there were significant (p < 0.05) decreases in the level of RBC, HGB and PCV (valued at 36%, 24% and 25% respectively) compared to normal control. However, these decreases were significantly (p < 0.05) increased after 6 h NNN post envenomation relative to the normal control (Table 5). The increment corresponds to 36%, 32% and 30% respectively. However, these increases were significantly reduced by 7–24% when cotreated with KV at 200 and 400 mg/kg compared to envenom group.

In the kidney of the envenom group, CRE, URE and UA levels were significantly (p < 0.05) elevated after 6 h NNN post envenomation relative to the normal control (Table 5). The increment corresponds to 36%, 32% and 30% respectively. However, these increases were significantly reduced by 8–36% when cotreated with KV at 200 and 400 mg/kg compared to envenom group.

Envenom group also had a significant (p < 0.05) decrease in the concentration of Na and HCO₃⁻ (by 3% and 14% respectively) with concomitant increase (10%) in K concentration when compared to normal control (Table 6). In KV treated groups, these changes were found to be attenuated by 0.9–9% at 200 and 400 mg/kg body weight.

### 3.5. Kolaviron mitigates oxidant/antioxidant alterations in NNN venom-treated rats

Kolaviron mitigates NNN venom-induced alteration in oxidant/antioxidant system of rats hepato-renal tissue (Table 7). The extent of lipid peroxidation was measured in the form of malondialdehyde (MDA) levels and significant (p < 0.05) increases of 33% and 39% were observed in the hepatic and renal tissue homogenates of envenom group compared to the normal control. But, in KV treated groups, the levels were found to be attenuated by 8–30% at 200 and 400 mg/kg. Additionally, there were significant (p < 0.05) increase in the SOD and CAT activities of hepatic tissues (35% and 30% respectively) and renal tissues (23% and 21% respectively) of envenom group relative to normal control group. These increments were attenuated by 4–35% in KV treated groups at 200 and 400 mg/kg.

### 3.6. Kolaviron reduces damage in the hepato-renal tissue of NNN venom-treated rats

Histological examination of NNN venom-induced hepatic (Fig. 3) and renal (Fig. 4) tissue damage mitigated by KV in rats. Histological

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### Table 2

Values for determination of NNN venom lethality in rats.

| Group | Dose (mg/kg) | Log₁₀ Concentration | Total No. of Rats | Number of Death | Percentage Mortality | Probit Value |
|-------|--------------|----------------------|------------------|----------------|----------------------|-------------|
| A     | 0.00         | –                    | 10               | Nil            | Nil                  | –           |
| B     | 0.30         | -0.523               | 10               | 2              | 20                   | 4.16        |
| C     | 1.30         | 0.114                | 10               | 5              | 50                   | 5.00        |
| D     | 2.30         | 0.362                | 10               | 7              | 70                   | 5.52        |
| E     | 3.30         | 0.519                | 10               | 10             | 100                  | –           |

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### Table 3

Effect of Kolaviron (KV) on some hematological parameters of NNN envenomed rats.

| Groups / Parameter | RBC (10¹²/L) | HGB (g/dL) | PCV (%) | WBC (10⁹/L) | NEUT (10⁹/L) |
|--------------------|--------------|------------|---------|-------------|--------------|
| Normal control     | 7.65         | 14.13      | 43.89   | 4.29        | 1.23         |
| Envenom (0.57 mg/kg) | ± 0.27a | ± 0.30b | ± 0.70c | ± 0.16d | ± 0.15e |
| Venom + KV         | 5.64         | 11.36      | 35.03   | 16.38       | 11.49        |
| 200 mg/kg          | ± 0.20f | ± 0.32g | ± 0.90h | ± 0.25i | ± 0.25j |
| Venom + KV         | 5.97         | 12.02      | 36.16   | 15.54       | 10.18        |
| 400 mg/kg          | ± 0.25k | ± 0.25l | ± 0.74m | ± 0.21n | ± 0.37o |

Values were expressed as mean ± SD (n = 6). Values with different letters (a,b,c,d) across the row are significant at P < 0.05. RBC = Red blood cell count; HGB = Hemoglobin concentration; PCV = Packed cell volume; WBC = White blood cell count; NEUT = Neutrophils

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### Table 4

Effect of Kolaviron (KV) on liver function indices of NNN envenomed rats.

| Groups / Parameters | AST (U/I) | ALT (U/I) | GGT (U/I) | TB (mg/dL) |
|--------------------|-----------|-----------|-----------|------------|
| Normal control     | 18.22     | 11.52     | 1.85      | 0.22       |
| Envenom (0.57 mg/kg) | ± 0.30d | ± 0.18e | ± 0.06f | ± 0.02g |
| Venom + KV         | ± 0.40a | ± 0.22b | ± 0.18c | ± 0.01d |
| 200 mg/kg          | ± 0.22a | ± 0.15b | ± 0.01b |
| Venom + KV         | 33.86     | 16.82     | 2.80      | 0.33       |
| 400 mg/kg          | ± 0.25a | ± 0.15b | ± 0.10c | ± 0.01d |

Values were expressed as mean ± SD (n = 6). Values with different letters (a,b,c,d) down the column are significant at P < 0.05. AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyl transferase; TB = Total bilirubin

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### Table 5

Effect of Kolaviron (KV) on kidney function indices of NNN envenomed rats.

| Parameters / Groups | Normal Control | Envenom (0.57 mg/kg) | Venom + KV 200 mg/kg | Venom + KV 400 mg/kg |
|--------------------|----------------|----------------------|----------------------|----------------------|
| Creatinine (mg/dL) | 1.05           | 1.64 ± 0.02a         | 1.50 ± 0.01b         | 1.34 ± 0.02c         |
| Urea (mg/dL)       | 25.27          | 37.35 ± 0.20a        | 34.80 ± 0.22b        | 30.80 ± 0.20c        |
| Uric acid (mg/dL)  | 2.88           | 4.10 ± 0.04a         | 3.80 ± 0.02b         | 3.30 ± 0.03c         |

Values were expressed as mean ± SD (n = 6). Values with different letters (a,b,c,d) across the row are significant at P < 0.05.

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### Fig. 2

Probit curve for determination of NNN venom LD₅₀ in rats.

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### Table 4 depict the effect of KV against NNN venom-induced liver dysfunction. Our results revealed that NNN venom induced significant (p < 0.05) increase in the level of AST, ALT, GGT, and TB (valued at 58%, 50%, 51% and 50% respectively) compared to normal control. But, in KV treated groups, the levels were found to be attenuated by 0.9–9% at 200 and 400 mg/kg body weight.
section of rat liver stained with haematoxylin & eosin (H&E) and examined under a light microscope (X100), showed normal morphology of central veins (CV), hepatocytes (H) and sinusoid (S) in control group (Fig. 3A). On the contrary, the hepatic tissue of envenom group showed a severe pathological lesion characterized by inflammatory (I) cells infiltration lar, vascular congestion (VC), condensed nuclei (C), sinusoidal dilation (SD), hepatocellular necrosis (HN) and apparently contained more kupffer (K) cells (Fig. 3B). However, these pathological changes were partially reduced in animals cotreated intraperitoneally with 200 mg/kg of KV (Fig. 3C) and moderately normalized in 400 mg/kg of KV treated groups (Fig. 3D).

4. Discussion

Envenoming by Naja species are well known to induce multiple toxic effects such as neurotoxicity, cytotoxicity and haematotoxicity [7,8]. These effects are associated with induction of oxidative stress which consequently plays an important role in the pathogenesis of tissue injury [9,30]. Unfortunately, anti-snake venom (the only available treatment...
for snakebite envenoming) fails to neutralize some of these effects [12]. In this study, kolaviron (a powerful antioxidant compound) has shown an effect on haematological abnormalities and hepato-renal toxicity, but its effects on neurotoxicity and blood haemostasis have not been assessed by this study, even though kolaviron might be effective against these complications.

Prior to investigation, LC-MS analysis was employed to confirm the identity of kolaviron via its components namely; Garcinia biflavonoid 2, Garcinia biflavonoid 1 A & B, kolaflavanone, binaringenin and kolaflavones. This corroborates the report of Okafor and Onyike [1]. Also, LD₅₀ of NNN venom was established intravenously in rat and found to be 1.14 mg/kg. Our LD₅₀ value differs slightly from that of Adeyi et al. [31] which reported 1.00 mg/kg via intraperitoneal route, but the signs and symptoms remained the same. The study of Fatima and Fatah [32] suggests that different route of administration could contribute to variations in snake venom lethality even among the same species. In the present study, haematological abnormalities and hepato-renal damage were observed in rats that received a single intravenous dose of NNN venom (0.57 mg/kg; \( \approx \text{LD}_{50} \)) after 6 h.

Snake venom comprises of many proteins that affect the vascular system especially the circulating blood cells [33]. In our study, we observed marked decrease in the RBC, HB and PCV levels in the venom-alone treated rats; an indication of haemolysis, due to venom effects on the erythrocyte blood cells. In the whole blood, erythrocytes are the most common targets for snake venom-induced toxicity. This is because their membranes are rich in unsaturated lipids; which happens to be highly preferred substrates for the venom cytotoxic PLA₂, leading to oxidative damage and haemolysis [9,34]. Since KV does not possess haematopietic properties, mitigation of NNN venom-induced haemolysis by KV might be attributed to its antioxidant capacity [35,36], thereby reducing the oxidative damage to erythrocyte cells. The principal role of leukocyte is to defend the body against foreign invading microorganisms or xenobiotics through phagocytosis and respiratory burst/ROS. Envenomed rats displayed leucocytosis, characterized by high neutrophil numbers (up to 9-fold increase), which could indicate infiltration of inflammatory cells [31]. However, these alterations were attenuated dose-dependently, by co-administration of KV at 200 and 400 mg/kg, suggesting that KV possesses substantial antioxidant capacity similar to previous study [36].

Hepatotoxicity and nephrotoxicity constitute most of the common and serious consequences of cobra snake envenomation [31]. Several factors contribute to the development of these alterations among which is the generation and production of tissue destructive free radicals such as highly reactive lipid peroxides by snake venom cytotoxin and PLA₂ [37,38,8]. MDA is generally considered as an excellent indicator of lipid peroxidation (LPO) and oxidative stress. Consistent with these studies [10,39], NNN venom administration in our study led to the elevation of MDA content in both hepatic and renal homogenates. This evidence suggests that NNN venom has a potent oxidative activity, which probably triggers ROS production and consequentially cause damage to the cells, tissues and organs [40]. Additionally, primary antioxidant enzymes such as SOD and CAT were evaluated. Envenom group showed a moderate increase in the activities of SOD and CAT. These could be considered as an adaptive response or a compensatory mechanism that enables the cells to overcome venom-induced damage [31]. However, these changes in both hepatic and renal homogenates were attenuated dose-dependently, by co-administration of KV at 200 and 400 mg/kg, suggesting that this compound possesses powerful natural antioxidant capacity as previously reported [18,41].

In the present investigation, we also explored certain parameters, considered as important and reliable predictors of hepatic and renal function. Similar to these studies [39,42], envenom group increased the serum level of certain liver function indicators e.g., AST, ALT, GGT and TB, which suggests hepatotoxicity, probably due to increased permeability of the cell membrane and/or breakdown of hepatic cells [43,44] by venom component. Co-administration of KV prevented the increase, thus revealing hepatoprotective nature of KV [18] against NNN venom-induced hepatotoxicity. In the case of kidney, envenom group increased the serum level of creatinine, urea, uric acid, and potassium with concomitant decrease in sodium and bicarbonate level. We can

![Fig. 4. Photomicrograph of kidney histopathology (H&E, X200). A. Kidney of control rat shows normal morphology of the glomerulus (GL), proximal (PT) and distal convoluted (DT) tubules. B. Kidney of rat after 6 h post envenomation of NNN venom (0.57 mg/kg; \( \approx \text{LD}_{50} \)) shows abnormal glomerular structures, infiltration of inflammatory cells (I) along inter-tubular spaces, renal tubular dilation (D) with the presence of blood, cortical renal tubular necrosis (N) and granular tubular casts (C). C. Kidney of rat co-treated with 200 mg/kg of KV shows mild mitigation of lesion with few abnormal structures. D. Kidney of rat co-treated with 400 mg/kg of KV shows moderate mitigation with very few abnormalities.](image-url)
assume that the observed changes in serum constituents could signify disturbances in renal function as a result of increased vascular permeability and oxidative damage [45–47] by the toxic action of snake venom components. In line with previous studies [19–21], KV strongly attenuates these alterations, thus showing substantial protection bestowed to rats against NNN venom-induced nephrotoxicity.

Furthermore, envenom animals showed histological evidence of tissue injuries, confirming the main clinical profile [48]. In our study, ultrastructural alterations induced by NNN envenomation were examined in liver and kidney tissues. According to Mukherjee and Maity [38], the progression of hepatocellular damage begins with disorganisation of cell membrane permeability, in this case by venom cytotoxin and PL-A2. Similar with this study [39], NNN venom stimulate infiltration of inflammatory cells leading to vascular congestion, condensed nuclei, sinusoidal dilation and hepatocellular necrosis. Hypertrophied von-Kupffer cells, observed in the study, indicate the phagocytic action of the hepatic tissue as a defence response to cell injury against envenoming [49,50]. Interestingly, co-administration of KV mitigated these lesions to an extent, and almost normalized at higher dose. These suggest that KV is probably effective at protecting the liver against the harmful effect of NNN envenomation.

For kidney, the glomerular and renal tubule epithelial cells are potential targets for numerous nephrotoxic agents like venom components [10]. In line with these studies [45,51], NNN venom component directly or indirectly disturbed the membrane integrity [52] leading to abnormal glomerular structure, and tubular lesions accompanied by invasion of inflammatory cells to the intertubular tissues in attempt to minimize the injury/or defend renal tissue [51]. The appearances of cytoplasmic vacuolation in the renal tubules suggest disturbances in lipid inclusions and fat metabolism occurring under pathological cases [53]. However, co-administration of KV attenuated these lesions to some extent, suggesting its ability to protect the kidney from destructive effect of NNN venom.

In conclusion, the finding of this study suggests that kolaviron could alleviates haemato logical abnormalities and hepatorenal damage caused by NNN venom treatment in rats through depletion of ROS and/or boosting the antioxidant system. Kolaviron itself might complement or substitute anti-snake venom (the only efficient therapy for snakebite envenoming), which is expensive, scarce and limited in neutralizing snakebite secondary complication like tissue injury.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Author statement**

Azubuike I. Okafor, contributed to the conception, design, execution, interpretation of the study and drafted the paper. Ndika N. Ogban and Anthonia A. Odingewe contributed to the conception, design, execution, and interpretation of the study. All authors have seen and approved the final version of the paper and have agreed to its submission for publication.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data Availability**

Data will be made available on request.

**Acknowledgement**

The authors wish to thank Prof. M.S. Abubakar and Dr. Peter Ofilli, for their technical assistance and Mr. Julius Sunday Iyoma, for histological tissue processing.

**Appendix A. Supporting information**

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.10.003.

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